THE CULTIVATION OF THE TUBERCLE BACILLUS ON ARTIFICIAL MEDIA

By

Milton Mezoff

Morris F. Shaffer

Submitted in partial fulfillment of the requirements

for the degree of

BACHELOR OF SCIENCE

from the

Massachusetts Institute of Technology

1930

Course VII

Signature of Author.....

Certification of Department.....

May 28, 1930.

Professor A. L. Merrill,
Secretary of the Faculty,
Massachusetts Institute of Technology,
Cambridge, Massachusetts.

Dear Professor Merrill:

The following thesis is submitted as a partial fulfillment of the requirements for the Degree of Bachelor of Science.

Respectfully submitted,

ACKNOWLEDGEMENT

The authors wish to express their thanks and appreciation for the assistance given them by Professor S. C. Prescott and Professor F. H. Slack. They also wish to thank the various individuals who were kind enough to send them cultures of tubercle bacilli.

PREFACE

The story of the development of the title of the thesis may be of some interest.

Originally one of the authors had planned to do as a bachelor's thesis a series of experiments testing the efficiency of pasturization in the destruction of vitality of the tubercle bacillus under varying conditions of time and temperature.

Consideration of the matter by the two present authors lead to a decision to do this work together; the subject was considered to be a favorable one, but the question of the development of suitable methods and technique arose. During a lecture in the course in Public Health Laboratory Methods, Dr. Slack had mentioned a new medium for the cultivation of the tubercle bacillus which had appeared from the Denver National Jewish Tuberculosis Sanatorium, as well as the use of an egg medium for this purpose which he felt would be of interest to the class.

The above references made a distinct impression in the minds of the authors as well as an article which they chanced to read in the Journal of Infectious Diseases.

In this article by Novy and Soule, the question of the respiration characteristics and requirements of the tubercle bacillus had been considered; valuable data and interesting theories were advanced.

As a result of the above combination of circumstances, it was decided to modify the thesis subject to deal with the formulation of a new method for the isolation of tubercle bacilli from various sources. Before this could be done, however, it was necessary that the authors should have familiarity with the problem, the necessary technique, and the growth characteristics of the organism.

It was finally decided that a preliminary study of the cultivation of the tubercle bacillus on artificial media would be necessary, and for the above reasons this subject was chosen for the thesis which follows.

As this work was the initial intimate contact of the authors with this vast problem there will, perhaps be found much therein which will not be corroborated by further investigation. Nevertheless we feel that our objective, a comprehensive investigation, by experiment and search of the literature, of the effects of physical chemical and biological forces on the growth characteristics of the tubercle bacillus, has been attained.

INTRODUCTION

The problem of the cultivation of the tubercle bacillus is one which has occupied the attention of a large group of capable and experienced investigators since the discovery of this organism as the causative agent of tuberculosis by Robert Koch in 1881. (1) Although in the present study no attempt was made to go beyond the consideration of the growth of the organism it may be interesting to delve into the motives which inspired many investigators in grappling with this highly absorbing question.

By cultivation of the microorganism, particularly on artificial media, it was hoped that much might be accomplished toward the develpoment of measures for specific therapy and prophylaxis, perhaps even as successful as those in other fields. The hopes of workers have not thus far been realized with respect to specific therapy. Attempts at prophylactc immunization through the use of attenuated or non-virulent cultures obtained after continued growth of the organism on special types of artificial media have been much more ambitious, notably in the case of Calmette and his co-workers, through the use of B.C.G. Even here, however, grave doubts have been cast upon the value of the results obtained, and it seems still too early to hope for the eradication of the "white plague" by this means.

By study of the physiology of the organism, more especially its

nutritional and metabolic requirements, it was believed that information would be obtained which would be of value in the treatment of cases of the disease. For example, it having been found that on artificial media the tubercle bacillus is glycerophilic, that is, glycerol is extremely desirably if not actually essential to the nutrition of the organism, subsequent experiments were performed on laboratory animals. Here the interesting fact developed that the inoculation of glycerol into an experimental animal leads to a lowering of bodily resistance with a tendency toward the formation of tubercles near the site of maximum glycerol concentration, as the organism grows better in the presence of this compound. Attempts at lowering the glycerol content of the living tissues, on the other hand, have not been so successful, but here too the indications are that diminished glycerol concentrations are favorable to an increase of resistance on the part of the host.

More recently, the question of cultivation has come to the fore, since by direct cultivation of the organisms from infected materials it has been claimed that diagnosis can be made more expeditiously (more rapidly, more accurately, and more cheaply) than by the older guinea pig inoculation method and more accurately than by the study of stained smears.

The work of investigators in this field has resulted in the accumulation of a large amount of valuable information not alone with regard to the tubercle bacillus but also to other members of the bacteriological world which have presented unusual difficulties of cultivation. Investigations of the cultivation of tubercle bacillus on artificial media may possibly lead to a better understanding of its relationship to other acid-fast organisms. Probably the most important direct utilization of facts gained from the study of the problem is in the case of the work being done under the auspices of the National Tuberculosis Association. Parke, Davis & Co., and H. K. Mulford Company are now engaged in the cultivation of the bacillus on media of defintely known composition. These media, accurately prepared and controlled, were devised by E.R.Long of the University of Chicago. As a result of the cultivation of the organism in large amounts (1000 or more Erlenmeyer flasks in a batch) the above groups hope to be able to learn something of the nature of growth and the body chemistry of the bacillus of Koch, since each batch is carefully analyzed chemically; the fractions separated and determinations made.

HISTORICAL

Koch, himself, was able in 1884, after many unsuccessful attempts, to grow the organism in pure culture by smearing crushed freshly caseous tubercles (from experimental animals) over coagulated, sterilized beef serum. He was also able to obtain scanty growth of the bacillus on sterile fluid serum, but was unable to grow it on broth or on nutrient agar. He concluded that "it is not to be hoped that the cultivation of the tubercle bacillus is to play any important part in the study of the disease."

In 1887, however, Nocard and Roux published in the Annals of the Pasteur Institute an article of fundamental importance, giving the results of their researches with the tubercle bacillus. These workers found that the addition of glycerin to various media such as broth, agar, or serum, in amounts varying from five to eight percent, greatly enhanced the value of the media for the cultivation of the tubercle bacillus.

The next year another investigator, A. D. Pawlowsky, also at the Pasteur Institute, described in the Annals of the Institute a method for the cultivation of the bacillus on glycerin potato.

These discoveries gave marked impetus to the conduct of investigations on the growth of the organism and the next few years saw the various scientific journals almost flooded with accounts of media and enraptured descriptions of wonderful results which might be obtained from their use. Many of these articles were devoted to the synthetic media composed chiefly of pure crystalline compounds. A large proportion of these were not worked out with sufficient care, as each one differed considerable from all others in the statements pertaining to the elements which were absolutely essential to the continued proliferation of the causative agent of tuberculosis. Later work, particularly that carried on during the second and third decades of the twentieth century seemed to indicate a more scientific experimental method of attack; and more accurate results were reported.

For each new medium which appeared, there soon followed a myriad of modifications, minor or important; each investigator changing the media to suit his fancy and reporting results on this basis. In consequence, any comparative studies which might be made lost a great deal of their significance.

The use of egg media for carrying along transplants of stock cultures of tubercle bacilli by Dorset, Lubernau, Besredka and others resulted

in the use of a modified egg medium by Petroff in 1915 for the routine cultivation of the organisms from sputum. Subsequent workers made changes in the formula of Petroff, notably Williams, and Burdick, Despeignes, and Corper, Fiala, and Kallen. Among the other media which have received considerable use in the routine cultivation of organisms from sputum as an aid to diagnosis are the media of Petragnani and Sweany and Evanoff. The potato medium of Corper has been widely used and with good results.

Cultures Used.

The following cultures, obtained through the courtesy of Dr. S. C. Prescott and various laboratories, were used at one time or another during the course of the research.

1. Tubercle bacillus, human strain.

   (a) Culture of the well-known H37; obtained from the Mass. State Antitoxin Laboratory at Forest Hills, Mass. The original culture came on a glycerol agar slant.

It is interesting to note that this widely used strain was isolated from sputum at Saranac Lake in 1905. There are now various substrains of this original strain.

(1) Phipps strain- used at the Henry Phipps Institute. This is reported as only slightly virulent.

(2) Novy strain-this was virulent four or five years ago, but is now innocuous as the result of very rapid growth and frequent transplanting under the conditions to which it has been subjected, namely cultivation on glycerin agar medium and closure with holed sealing wax.

(3) Trudeau strain-used at the Research and Chemical Laboratory at Trudeau, N.Y. This is

This is fully virulent. (Mudd,S. and Furth,J Journal of Immunology--V.13 No.5 May 1927)

The culture obtained from the State Antitoxin Laboratory was not identified as belonging to any of these sub-strains, but inoculation of organisms from this culture into a guinea-pig demonstrated it to be virulent.

(b) Culture of "54"; obtained from the Albany Medical College, Albany, N.Y. The original culture arrived on a gentian-violet potato slant. This culture had a distinct yellow-orange chromo-genicity. S ubsequent cultivation demonstrated very rapid growth, while guinea pig inoculation showed it to be non-virulent at the time.

(c) Culture of "Be"; obtained from the New York State Agricultural College, Cornell University, Ithaca, N. Y. This culture also arrived on a glycerol agar slant.

(d) Culture of "G624"; obtained from the Michigan State Antitoxin Laboratory at Lansing, Mich. This culture was originally on a potato slant. The labels on the cultures of "G624" bore the legend, "Isolated from Spinal Fluid".

(e) Culture of "S1800"; obtained from the Mulford Laboratories, Philadelphia, Penn. The culture was growing on a glycerol agar slant.

2. Tubercle bacillus, bovine strain-

(a) Culture of "1689", also from the Mulford Laboratories. This culture, too, was grown on glycerol agar.

(b) Culture of "Traum271"; obtained from the New York State Agricultural College, Cornell University, Ithaca, N.Y. This culture, also, arrived on a glycerol slant.

The cultures arrived within a few days of one another and were placed in the refrigerator immediately upon arrival, until ready for use.

In order to simplify the future processes of classification and indexing, the different cultures were coded as follows:-

"H37"	was	given	code	letter	H
"54"	2	"	"	"	Z
"Be"	"	"	"	"	B
"G624"	"	"	"	"	G
"S1800	"	"	"	"	S
"1698"	"	"	"	"	D
"T271"	"	"	"	"	T

Following the code letter there appeared on the labels and in the record book a number, indicating the identity of the inoculation and the nature of the medium on which the inoculation was made. For example:-

H8. signified that the inoculation was made with a culture or transplant of "H37", that it was the eight inoculation made, and reference to the record book would reveal the information that the medium used was medium B (Hease's Agar).

Technique Employed

It seems worthwhile to record here the most important methods of technique employed during the course of this work in order that some idea may be obtained as to their shortcomings as well as their value.

Inoculation:

Fairly stiff inoculating needles were used. One of us (Mezoff) preferred a 12 guage platinum wire mounted in a special handle. The free end of the wire was bent in the form of a loop 2 mm.. in diameter. The other(Shaffer) preferred a nichrome wire mounted in an aluminum handle; the nichrome needle, while slower to cool after heating in the flame, was stiffer and therefore perhaps a trifle more suited for inoculations.

For inoculating on solid media the inoculum was in all cases smeared as smoothly as possible(at times with great difficulty) over the surface of the medium. For inoculations into liquid media the technique was somewhat modified, when the inoculum was to be floated on the surface of the medium. The flask was allowed to remain on the workbench; the plug or stopper was removed, the opening flamed, then the inoculation quickly made, on the side of the flash, above the level of the liquid. Then the flask was slanted so that the liquid touched the inoculum, after which it was allowed to come to rest on the desk as before.

Then the plug was flamed together with the mouth of the flask and the plug returned. Every effort was made to prevent contaminations which were a serious problem in some cases.

In order to prevent dessication of the media the following technique was adopted:-

1. The exposed ends of the cotton plugs which closed the mouths of the tubes were flamed, the flames quickly extinguished by gentle dry blowing, and the plugs pushed into the tubes until their upper surface was about one quarter inch below the mouth of the tube. Then, rectangular prisms of paraffin, about $\frac{1}{4}$" thick and slightly larger in area than the mouth of the tube having been prepared, these were pressed firmly into the mouths of the tubes so that they were completely sealed. When the tube was thus completely sealed, a stiff inoculating needle with a spatula tip was heated and introduced into the paraffin to create a small opening from the exterior thru the paraffin to the cotton. In some cases two holes near the edge of the tube were made, in some cases only one hole. In still other cases, the tubes were not sealed with paraffin at all, but merely with the cotton plug. In such cases there was some tendency to dessication of the medium. The holes in the paraffin were for the purpose of permitting slow diffusion and exchange of gases, while the rate of dehydration of the media was retarded. Absolute wax seals were not used, since Novy points out that the use of absolute seals inhibits the growth of the culture.

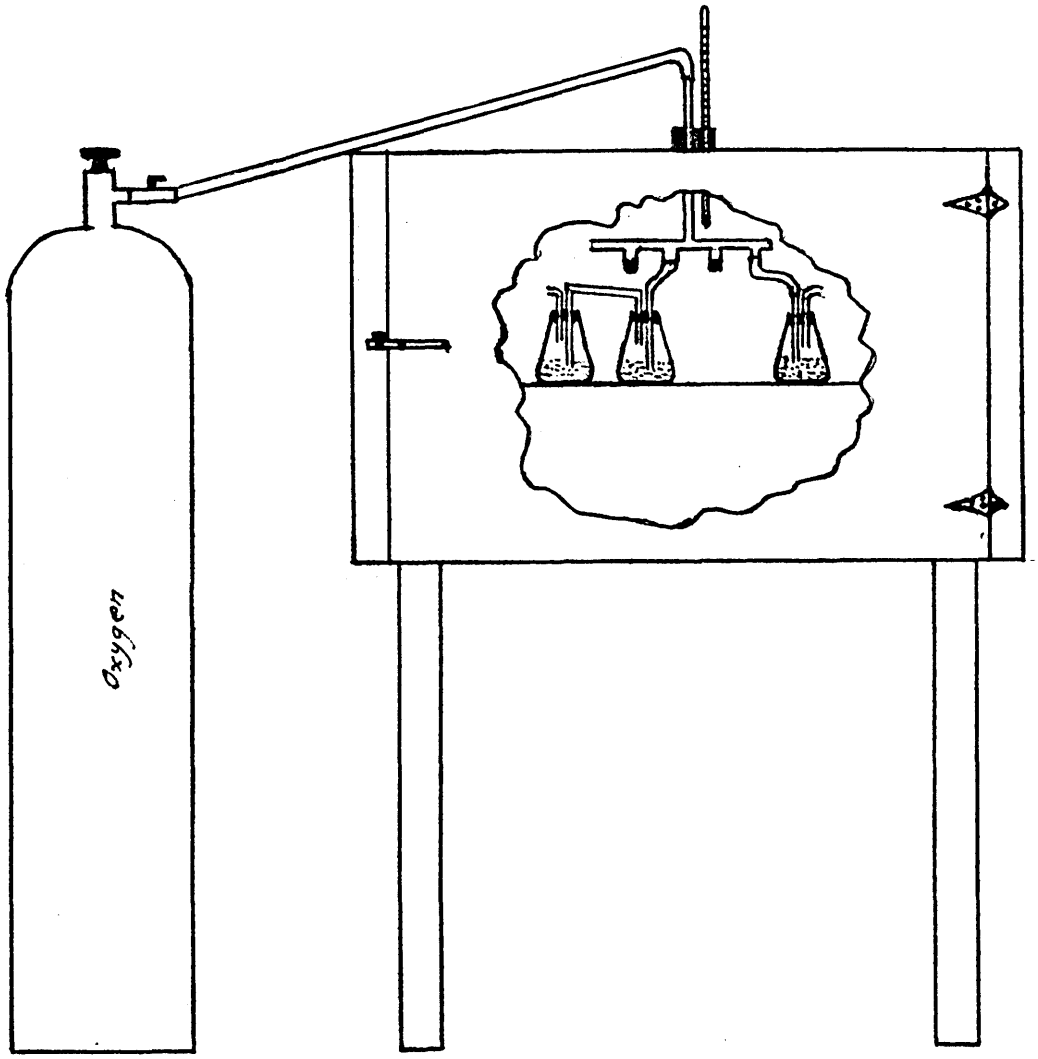
Oxygenation:

For oxygenation of liquid media cultures, a special apparatus was constructed. Fig. 1

A tank of compressed oxygen equipped with a reduction valve from the nozzle of which the oxygen was conducted by means of rubber tubing through a hole in the top of the incubator, to a hollow glass "cake" in the incubator. This glass cake had a single inlet, but a number of outlets. From the outlets, rubber tubing conducted the oxygen to the flasks. The flasks were ordinary Erlenmeger flasks, stoppered with two-holed rubber stoppers. A right angle glass bend running through the stopper, but not touching the medium, served to conduct the gases to the exterior. Both bends were loosely plugged with cotton to prevent contamination in so far as possible. The flasks, stopper and glass bends were sterilized together before using. In use, the oxygen passed through the rubber tubing into the incubator, into the "handle" of the glass cake, through one of the prongs along rubber tubing into the flask, down into the medium, bubbling up through, a portion of the oxygen dissolving in the medium during this period. The gaseous pressure at the surface of the medium was relieved by the second glass bend which conducted gases to the exterior. The flasks might also be arranged in series, with the outlet of one connected to the inlet of another, so that the excess gases (containing a high percentage of O) might be utilized further. With the nozzle of the tank adjusted properly, oxygen could be bubbled through the medium at the rate of 1,2,3,4,5, or more bubbles 1 second (the orifice of the inlet tube having a diameter of 5 mm.,) so that the medium might

be kept completely saturated with dissolved oxygen at 37°C over considerable periods of time. This method did not yield very good results in our work, but we recommended its use to another worker(Tobie) who found it increased the growth and pigment production of B. violaceus several times, enabling the pigment and organisms to be produced throughout the medium instead of merely at the air-medium interface.

Figure 1



Experiment I

The use of glycerol agar in the cultivation of the tubercle bacillus may be traced back to the earliest investigators. This perhaps was due in large part to the simplicity of the composition of the medium. It is rather difficult to ascertain just who was the first to use glycerol but as far back as 1887 Nocard and Roux employed it in media(broth, agar, or serum) containing 5 to 8 percent of glycerol. (The question as to the necessity of glycerol in this medium is discussed in Expt. 2) Glycerol agar is very useful for making transplants to maintain the viability of the organism; it will be remembered that all the cultures used excepting G624 and "54" in this and the following experiments, were received from various scources on glycerol agar. This medium is also useful, due to its definite and simple composition, in cases where it is desired to determine the effect of various substances on the growth of the organism, these substances being added to the glycerol agar. Dostal in 1916 subjected pure cultures of tubercle bacilli to chemical influences. He added to the ordinary glycerol media 5 to 10 parts by weight of a glucoside, saponinum deprivatum Merck. Loewenstein in 1911 and later Lockemann worked with media containing simple inorganic salts plus glycerol in order to determine the essential elements for growth.

Goodman and Moore found that the human tubercle bacillus grows equally well on culture tubes containing slants of glycerol agar or egg medium (Petroff's), regardless of whether the tubes have been capped with waxed cloth or with tin foil, securely held in place with rubber bands, thus indicating a ready access of atmospheric air in the folds of the tin foil, in spite of precautions to prevent this means of escape of the carbon dioxide produced by the respiration of the bacilli.

With regard to the effect of the medium on the chemical composition of the bacillus, Long and Finner found that the glycerol (in Long's synthetic medium) promotes acid-fastness and yield, and is regarded as a progenitor of the waxy constituents of the bacillus. The lipin content is increased only with high concentrations of glycerol.

In order to obtain enough material for later experiments involving inoculations, to maintain the life of the cultures received, and to determine whether or not the various strains of tubercle bacilli used could be grown on the ordinary stock nutrient agar to which three percent glycerin had been added, the procedure given below was performed.

Primary transplants from the original cultures received were made onto slants of medium A (3% glycerol agar) and observations of the growth obtained. In sealing the tubes three methods were tried, as follows:

(a) Cotton plugs alone.

(b) Cotton plugs were flamed at the ends, extinguished, then pushed down into the neck of the tubes. The mouths of the tubes were then sealed with paraffin which was punctured with the aid of an inoculating needle to give (1)paraffin seals with one hole, and (2) paraffin seals with two holes.

The tables (1-7) on the following pages contain the observations made on the transplants treated in the manner just described. The composition of the medium used is also given.

As will be seen by inspection of the tables nearly all the tubes showed some signs of growth except in the case of G624 and 271. The failure of these trains, isolated from spinal fluid, and bovine sources respectively to show growth in three-fourths of the tubes inoculated, may perhaps be attributed to the age and condition of the original culture; they presented a dessicated appearance when received by us. On the other hand, strain 54

showed the presence of considerable growth in two weeks.

Although the cultures required more time for exhibiting growth than we had anticipated, we were struck by the fact that for any given strain the cultures required unusually uniform length of time for exhibiting growth. In general the cultures required about seven or eight weeks before exhibiting considerable growth. The type of stopper does not seem to have affected the results very materially in any case. The appearance of the growths was fairly distinct for each strain, the chromogenicity varied from a light cream color to a fairly deep orange and in the case of H37 it will be noticed that there are two types of colonies, smooth and wrinkled.

MEDIUM A

3% Glycerol Agar

1. To 1000 cc. water (distilled) were added the following:-

   30g. glycerol
   8g. Nacl
   23g. Difco Bacto Nutrient Agar

   The ingredients were thoroughly dispersed in the water by boiling and stirring.

2. They were then made up to weight with hot water, filtered and tubed; 10 cc. to each tube.

3. The tubes were autoclaved thirty minutes at fifteen pounds pressure, then slanted and cooled.

4. The pH. determined immediately after autoclaving, was 6.8.

TABLE I

"S_{1800} Strain"

Date of Inoculation Feb. 5, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth
S_5	P*-1 hole	Feb. 14	Mar. 28	heaped up, wrinkled
S_6	" " "	Mar. 7	Apr. 15	contaminated
S_7	" " "	Feb. 14	Mar. 28	#1. small, flat white 2. heaped up, cream colored.
S_8	" " "	" "	" "	heaped up, wrinkled
S_9	" " "	" "	" "	" "
S_{10}	" " "	" "	" "	" "
S_{11}	" " "	Feb.24	Apr. 6	" "
S_{12}	" " "	"	Mar. 28	" "
S_{13}	" " "	Feb. 14	Mar. 28	" "

*Paraffin

#Possible dissociation

TABLE 2

"1698" STRAIN

Date of Inoculations Feb. 5, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth
D_5	cotton plug no P*	Mar. 14	Apr. 2	white, heaped up
D_6	P__ 1 hole	"	"	pale white heaped up
D_7	" "	"	"	wrinkled growth
D_8	" 2 "	"	"	white heaped up. wrinkled
D_9	" "	"	"	"
D_{10}	" "	no growth		
D_{11}	" "	Mar. 21	Apr. 24	wrinkled growth
D_{12}	" "	June 2		very slight

*Paraffin

TABLE 3

"G_{624} STRAIN"

Date of Inoculation Feb. 5, 1930

Tube No.	Type of stopper	Date of first appearance of growth	Approximate date of presence of considerable growth	appearance of growth
G_3	P--1 hole	no growth (June 2)		
G_5	" " "	"	"	
G_6	" " "	"	"	
G_7	P--2 hole	Apr. 14	May 21	creamy, heaped up wrinkled, button-shaped
G_8	" " "	no growth (June 2)		
G_9	" " "	"	"	
G_{10}	" " "	"	"	
G_{11}	" " "	Apr. 4	May 21	creamy, heaped up wrinkled, button-shaped

TABLE 4

"54" STRAIN

Date of Inoculation Jan. 31, 1930

Tube No.	Type of stopper	Date of first appearance of growth	Approximate date of presence of considerable growth	appearance of growth
Z_7	cotton plug no P*	Feb. 7	Feb. 14	large, spreading orange, wrinkled
Z_8	"	Feb. 14	Feb. 21	" "
Z_9	P--1 hole	Feb. 7	Feb. 14	large, spreading orange, surface.
Z_{10}	" "	"	"	" "
Z_{11}	" "	"	"	" "
Z_{12}	" "	"	"	much surface growth some sub-surface.
Z_{13}	" "	"	"	spreading, orange wrinkled
Z_{14}	" "	"	"	"
Z_{15}	" "	"	"	"
Z_{16}	" "	"	"	"
Z_{17}	" "	"	"	"
Z_{18}	" "	"	"	"

TABLE 5

Be STRAIN.

Date of Inoculation Jan 31, 1930

Tube No.	Type of stopper	Date of first appearance of growth	Approximate date of presence of considerable growth	appearance of growth.
B_7	cotton plug no P*	Mar. 14	Apr. 7	yellow-brown heaped up wrinkled spreading.
B_8	" "	Mar. 7	Apr. 7	single large, knob-like
B_9	P--1 hole	Mar. 14	Apr. 7	large, spreading, heaped up
B_{10}	" " "	Mar. 21		slight growth
B_{11}	" " "	no growth June 2		
B_{12}	" " "	Mar. 7	Apr. 7	large, spreading, heaped up, wrinkled.
B_{13}	" " "	Mar. 7	Apr. 14	wrinkled, heaped up
B_{14}	P--2 hole	no growth June 2		
B_{15}	" " "	Mar. 21	Apr. 24	#1. small, isolated, flat 2. large, wrinkled, heaped up.
B_{16}	" " "	no growth June 2		
B_{17}	" " "	no growth June 2		
B_{18}	" " "	Mar. 7	Apr. 14	wrinkled, heaped up
B_{19}	" " "	Mar. 7	Apr. 7	wrinkled, knob-like

P*--Paraffin

Possible dissociation

TABLE 6

H37 STRAIN.

Date of Inoculations Jan. 3, 1930

Tube No.	Type of stopper.	Date of first appearance of growth	Approximate date of presence of considerable growth	Appearance of growth
H_{10}	cotton plug no P*	Feb. 14	Mar. 7	heaped up, wrinkled colonies
H_{11}	" "	Feb. 24	" "	heaped up, wrinkled
H_{12}	P--1 hole	Feb. 14	Mar. 14	" "
H_{13}	" " "	" "	Mar. 21	small, flat, white. spreading, wrinkled, heaped up, cream-colored
H_{14}	" " "	Mar. 14	Mar. 21	heaped up, wrinkled
H_{15}	" 2 Hole	Feb. 14	Mar. 7	1 white, flat surface 2 small, moist, separate, heaped up.
H_{16}	" " "	" "	" "	moist, heaped-up
H_{17}	" " "	Feb. 24	" "	" "
H_{18}	" " "	Feb. 14	" "	" "

TABLE 7

"271" STRAIN

Date of Inoculations Jan. 31, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth
T_4	cotton-plug-no P*	no growth June 2		
T_5	"	"		
T_6	P--1 hole	"		
T_7	" " "	"		
T_8	" " "	"		
T_9	" " "	Mar. 14		slight growth
T_{10}	" " "	"		" "
T_{11}	" " "	"		" "
T_{12}	" " "	no growth		
T_{13}	" " "	" "		
T_{14}	" " "	Mar. 14		slight growth
T_{15}	P--2 hole	no growth June 2		
T_{16}	" " "	"		
T_{17}	" " "	"		
T_{18}	" " "	"		

Experiment II.

The Effect of Glycerol on the Growth of the Tubercle Bacillus.

Although glycerol is used in nearly all media composed specifically for the cultivation of the tubercle bacillus, the question as to the necessity for this substance and the concentrations in which it might be used still has some aspects of uncertainty.

Kendall, Day and Walker in 1914 in their first study of acid-fast bacteria make the following interesting statement, "This work is important from another point of view. Prior to the publication of these studies, investigators, almost without exception, assumed that tubercle bacilli could not be cultivated in artificial media unless glycerol was present. Some observers have gone so far as to state dogmatically that glycerol was sine qua non for the artificial cultivation of these organisms. The observations mentioned above show definitely that the majority of strains of tubercle bacilli will develope, although much less luxuriantly, even in plain nutrient broth. These organisms, therefore, are not obligately glycerophlic." In a later study (1920) these authors suggest that the development of an acid reaction by human bacilli together with minimal deamination, as shown by the decrease in the ammonia content of the medium during the period of growth, is due largely to the utilization of the glycerol for the energy requirements of the bacilli, yielding acid products in its breakdown, but shielding the protein constituents of the medium. That is, the latter

are broken down only as their nitrogen is needed for incorporation in bacillary substance, the glycerol sufficing for consumption to produce heat and energy.

An interesting study was made by Vaudremer in 1921 of certain strains of tubercle bacilli which grew on potato or gelatin without glycerol, and not only on broth but in it. Three such strains one of bovine and two of human origin, are described. The bacilli cultivated in potato broth grew in twenty-four hours at 37°C., were non-acid-fast, Gram positive, and had a morphological resemblance to the bacterioides of legumes. Their identity with tubercle bacilli was shown by a return to acid-fastness after ten days on glycerolated potato, and by their agglutination by the sera of tuberculous patients. However they were avirulent. On the other hand, in 1922 Gessard and Vandremer grew tubercle bacilli in distilled water plus 40% and tap water plus 5% of glycerol when a paper support was provided.

Frouin and Guillaumie in 1923 found that in the absence of glycerine the human type of bacillus tends to alkalinize the culture, but in these conditions the fat content diminishes from 60 to 80 percent. In a subsequent paper they found that the salts of iron increase growth is media containing sugar and glycerine whatever be the reaction of the medium, but if glycerine is lacking, growth is increased in an acid and diminished in a neutral medium by the addition of iron. Corper, Fiala and Kallon believe that on five percent glycerol agar, a good growth of tubercle bacilli was obtained within a wide

range of reaction of the medium. Another set of experiments showed that approximately equal results were given whether the glycerol percentage of the medium was 1,2 or 4 percent.

Experimental: This experiment was performed in order to repeat, and if possible, to substantiate the results of many other workers. The following tables depict the effects produced by varying concentrations of glycerol on the rate and amount of growth of the tubercle bacillus. The medium employed (Medium F.) did contain from 1% to 9% of glycerol. In certain instances no glycercol was added. Incubation was at 37°C. It is unfortunate that the cultures on this experiment suffered so severely from mold contamination. A repetition of the experiment, although highly desired, was impossible due to lack of time.

In the case of the cultures containing no glycerol growth was, with one uncertain exception, unattainable. In cultures containing glycerol contamination was so frequent that, with the exception of strain 54, no comparative studies can be made. It will be observed that even the higher concentrations of glycerol were unable to inhitbit mold formation. In case of strain 54, growth was rather profuse in media containing glycerol in concentrations up to 7%. In concentrations above 7% growth occurred but in decreasing amount.

MEDIUM B

Glycerol Agar

To 1000 cc. of distilled water was added 23g. of Bacto Nutrient Agar (dehydrated) together with 8g. of Nacl. The mixture was brought to a boil and then filtered. To the filtrate was added glycerol in varying amounts, the mixtures were autoclaved at 15# pressure for 25 minutes, then slanted and cooled. The pH was determined colorimetrically while the mixtures were hot. Distilled water was used in preparing the glycerol solutions.

F1--9cc.agar- 1 cc. of 10% glycerol solution --pH-- 6,9

F2-- " " " " " 20% " " --pH-- 6,9

F3-- " " " " " 30% " " --pH--6,8

F4-- " " " " " 40% " " --pH-- 6,8

F5-- " " " " " 50% " " --pH-- 6.7

F_6-- " " " " " 60% " " --pH-- 6.7

F7-- " " " " " 70% " " --pH-- 6.7

F8-- " " " " " 80% " " --pH-- 6.6

F_9-- " " " " " 90% " " --pH-- 6.5

TABLE 8

Nutrient Agar without Glycerol

Date of Inoculations Feb. 15, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth.
T_{31}	P*--1 hole	no growth in 10 weeks		
T_{32}	" " "	" " " " "		
B_{31}	" " "	" " " " "		
B_{32}	" " "	" " " " "		
Z_{31}	" " "	Mar.7		sparse, flat, yellow.
Z_{32}	" " "	" "		" " "
H_{31}	" " "	no growth in 7 weeks		
H_{32}	" " "	" " " "		
G_{31}	" " "	" " " 3 "		(mold contamination)
G_{32}	" " "	" " " " "		" "
D_{31}	" " "	" " " 5 "		
D_{32}	" " "	" " " " "		
S_{31}	" " "	" " " 6 "		
S_{32}	" " "	" " " " "		

*Paraffin

TABLE 9

(1% glyderol nutrient agar)

Date of Inoculation--Feb. 21, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth.	Appearance of growth.
T_{41}	p*--2 hole	no growth in 10 weeks		
Z_{41}	" " "	Feb. 25	Mar. 7	spreading, yellow
Z_{42}	" " "	" "	" "	" "
H_{41}	" " "	contaminated in 4 days		
H_{42}	" " "	"	" "	
G_{41}	" " "	"	" "	
D_{41}	" " "	"	after 2 weeks	
D_{42}	" " "	"	" "	
S_{41}	" " "	Apr. 15	May 11	heaped up, wrinkled
S_{42}	" " "	" "	" "	" " "

TABLE 10

(2% glycerol nutrient agar)

Date of Inoculations--Feb. 21, 1930

Tube No.	Type of stopper	Date of first Appearance of growth.	Approximate date of presence of considerable growth	Appearance of growth.
T_{43}	P*--2 hole	no growth in 10 weeks		
T_{44}	" " "	" " " "		
B_{43}	" " "	contminated by mold in 2 weeks		
B_{44}	" " "	" " "		
Z_{43}	" " "	Feb. 25	Mar. 7	spreading yellow wrinkled
Z_{44}	" " "	"	"	" " "
H_{43}	" " "	contaminated by mold		
H_{44}	" " "	"	"	
G_{43}	" " "	"	"	
G_{44}	" " "	"	"	
D_{43}	" " "	"	"	
D_{44}	" " "	"	"	
S_{43}	" " "	"	"	
S_{44}	" " "	"	"	

*Paraffin

TABLE 11

MEDIUM F_3

(3% glycerol nutrient agar)

Date of Inoculations--Feb. 21, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth.
T_{45}	P*--2 hole		contaminated by mold	
B_{45}	" " "		" " "	
B_{46}	" " "		" " "	
Z_{45}	" " "		" " "	
Z_{46}	" " "	Feb. 25	Mar. 1	spreading, yellow, wrinkled
H_{45}	" " "		contaminated by mold	
H_{46}	" " "		" " "	
G_{45}	" " "		" " "	
D_{45}	" " "		" " "	
D_{46}	" " "		" " "	
S_{45}	" " "		" " "	
S_{46}	" " "		" " "	

*Paraffin

TABLE 12

MEDIUM F_4

(4% glycerol nutrient agar)

Date of Inoculation--Feb. 21, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth.
T_{47}	P*--2 hole		contaminated by mold	
B_{47}	" " "		" " "	
Z_{47}	" " "	Feb. 24	Feb. 28	spreading, yellow wrinkled
H_{47}	" " "		contaminated by mold	
G_{47}	" " "		" " "	
D_{47}	" " "		no growth in 10 weeks	
D_{48}	" " "		" " " " "	
S_{47}	" " "		contaminated by mold	
S_{48}	" " "		" " "	

*Paraffin

TABLE 13

MEDIUM F5

(5% glycerol nutrient agar)

Date of Inoculations--Feb. 21, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of Growth
Z_{49}	P*--2 hole		contaminated by mold	
H_{49}	" " "		" " "	
D_{49}	" " "		" " "	
S_{49}	" " "		" " "	

MEDIUM F_6

(6% glycerol nutrient agar)

Date of Inoculations Feb. 21, 1930

T_{51}	" " "		contaminated by mold		
B_{51}	" " "		" " "		
Z_{51}	" " "	Feb. 27	Mar. 8		slight growth
H_{51}	" " "		contaminated by mold		
H_{52}	" " "		" " "		
G_{51}	" " "		" " "		
D_{51}	" " "		" " "		
S_{51}	" " "		" " "		

*Paraffin

TABLE 14

MEDIUM F_7

(7% glycerol nutrient agar)

Date of Inoculations--Mar. 7, 1930

All contaminated by mold except Strain54.

Z_{55} Mar. 14---sparse growth
diameter of growth is 5/8"
growth is sharply limited, oval in outline and granular.
Mar.21----diameter of growth is 3/4"
appearance same as above
no visible growth after Mar. 21

MEDIUM F_8

(8% glycerol nutrient agar)

Date of Inoculations Mar. 7, 1930

All contaminated by mold except strain 54.

Z_{58} P*--2hole Mar. 14 diameter of growth 1/4"
Mar. 21 " " " 3/8"
no further visible growth after this date.

MEDIUM F_9

(9 % glycerol nutrient agar)

Date of Inoculations Mar. 7, 1930

All contaminated my mold except strain 54.

Z_{60} Mar. 14--diameter of growth 1/4"
Mar. 21--diameter of growth 3/8"
appearance was similar to that of culture on 8% glycerol nutrient agar.

TABLE 15

MEDIUM F_{10}

(10% glycerol nutrient agar)

Date of inoculations--Mar. 7, 1930

All contaminated my mold except strain 54

Z_{62} Mar. 14---diameter of growth 1/2"
Mar. 21--- " " " 3/4" --- no further visible growth
appearance was imilar to that of cultures on 8% and 6% glycerol nutrient agar.

Experiment 3.

Glycerol Nutrient Broth

Glycerol nutrient broth has been used extensively where cultures in fluid media are required. It has been of great value in studies on the charges of the reaction of the medium produced by growth of the bacillus and in studies dealing with the products of the metabolism of the organism.

Frothingham in 1918 studied the reaction of the medium by means of litmus bouillon containing 5% of glycerol and brom cresol purple bouillon containing 3% of glycerol.

Petroff (1918) in preparing a glycerol extract of tubercle bacillifon antigen in complement fixation, grew the organism in 4% glycerol-beef-broth for from 4-6 weeks until the whole surface was completely covered with a thick pellicle of tubercle bacilli.

Kendall, Day and Walker employed glycerol broth in their important series of articles dealing with the metabolism of the tubercle bacillus and other acid-fast organisms.

Ishimori (1924) compared the range of growth of saprophytic acid-fast bacilli and the reaction changes produced in glycerol-bouillon with that of warm and cold-blooded tubercle bacilli. Pure eaprophytic strains grew well in strongly alkaline as well as strongly acid-media, while the genuine warm blooded tubercle bacilli had narrower ranges of growth. The reaction changes occurring in glycerol-bouillon during the growth were dependent on the hydrogen-ion concentration of the medium

at the time of inoculation and upon the individual strain. The reaction also influenced markedly the inhibitory action of chemical substances.

EXPERIMENTAL:

On January 28, 1930 an inoculation was made from the original slant of T_{271} into a 250 cc. Erlenmeyer flask containing 100 cc. of glycerol nutrient broth (Medium N) having pH of 6.8.

On Feb. 15 the medium had become turbid, while the seedings, which had increased in size, had fallen to the bottom of the medium. On Feb. 25 the following appearance was noted. The broth was turbid, and on the surface were a number of waxy patches and were (10-15 mm. in diameter.) These latter covered almost the entire exposed surface of the liquid and some were on the sides of the flask. In the center of the wafer-thin waxy patches and resting on them were oily-looking globules scattered over the surface. The patches themselves were grayish white (dull, waxy) and irregular in contour.

On Mar. 14 there were no longer any patches on the surface, there was much sediment in the flask and the broth was turbid.

MEDIUM C

Glycerol Nutrient Broth (Calmette--P.34)

Calmette says that this may be prepared by adding 4 to 5% of glycerol to well-cleared ordinary veal or beef peptone broth. The medium is also rendered weakly alkaline to litmus as indicator.

Our Modification (Medium C.)

Difco Bacto Nutrient Broth (contains 3 parts beef extract and 5 parts peptone)	8.0 g.
Glycerol	50.0 g.
Distilled water	1000.0 cc.

The dehydrated broth was dissolved in water. It was then filtered and the glycerol added with stirring; finally it was autoclaved at fifteen pounds pressure for twenty minutes and was then ready for use. It was used in 300 cc. Erlenmeyer flasks, about 100 cc. to the flask. The pH. was 6.8.

EXPERIMENT 4

Hess's medium

This experiment was carried out to determine the value of Hess's medium (medium B) as a "proprietary" substrate for the growth of the tubercle bacillus, in view of the claims which have been made for it. The same system of stoppering the tubes was used as in Experiment I. This medium showed considerable water of condensation at the bottom of the slants, whereas the agar slants of Experiment I showed none.

The procedure consisted in making transplants form the original cultures of the various strains onto slants of Hess's agar, and incubating at 37°C.

As is evident from the tabulated results on the following pages/that growth, while present, was far from being as rapid as other investigators have reported. In the case of G624 no growth was obtained but the strain also grew only with extreme difficulty on other media used in the experiments presented here so that its behavior in this instance was not unusual.

MEDIUM D

Hease's Medium (Calmette---P.46)

1. Heyden Nutrose	5g.
Glycerol	30g.
Sodium Chloride	5g.
Agar	10g.
Normal solution of NaOH (crystallized at 28.6 per hundred)	5cc.
Distilled water	1000cc.

Our Modification (Medium D.)

1. The directions called for a special preparation, which is prepared in Germany under the name, "Nahrstoff Heyden". In our medium we utilized nutrose obtained from an unopened package of "Lion" Brand Nutrose which was several years old, judging from appearances. The failure to obtain the desired growths in our experiments with this medium may be attributed in part to the deteriorated condition of this product.
2. In following the directions given in Hiss and Zinsser's "Text-book of Bacteriology" we used 10g. of nutrose as they stated, rather than the 5g. which Calmette says are required.

3. Instead of using NaOH, 5cc. of a 28% Sodium Carbonate solution were employed.
4. The nutrose, sodium chloride, glyderol, agar and sodium carbonate solution were added to the water and boiled for about ten minutes.
5. They were then filtered through cotton and tubed.
6. The medium was now autoclaved twenty minutes at 15# pressure, slanted and allowed to cool.

TABLE 16

Strain "1698" Date of Inoculations- Feb. 5, 1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approximate date of Presence of Considerable Growth	Appearance of Growth
D1	cotton plug-no P*	Mar. 4	May 5	small,flat,separate
D2	P-1 hole	Mar. 7	Apr. 4	" " "
D3	P-2 Hole	Mar. 7	Apr. 21	small, pale,white
D4	" " "	Mar. 8	Apr. 14	thin,flat,spreading growth-in middle is moist,heaped-up, pinkish growth

Strain S1800 Date of Inoculations- Feb. 5, 1930

S1	P-1 hole	Feb. 14	Apr. 2	distinct,knob-like wrinkled
S2	" " "	" "	" "	" " "
S3	P-2 hole	Feb. 24	Apr. 15	knob-like,heaped-up
S4	" " "	" "	" "	" " " "

*Paraffin

TABLE 17

Strain "54" Date of Inoculations- Jan. 31, 1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approximate date of Presence of Considerable Growth	Appearance of Growth
Z1	cotton plug-no P*	Feb. 7	Mar. 1	orange-yellow, spreading, wrinkled
Z2	" " "	" "	" "	" "
Z3	P-1 hole	" "	" "	" "
Z4	" " "	" "	" "	" "
Z5	" 2 "	" "	" "	" "
Z6	" " "	" "	" "	" "

Strain "G624" Date of Inoculations- Feb. 5, 1930

G1	P*-2 hole	no growth June 2	
G2	" " "	" "	
G3	"cotton" plug-no P	" "	

*Paraffin

TABLE 18

Strain "Traum 271" Date of Inoculations- Jan. 31, 1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approximate date of Presence of Considerable Growth	Appearance of Growth
T1	P*- 2 hole	Mar. 7		slight increase of inoculation
T2	" 1 "	no growth June 2		" " "
T3	cotton plug-no P*	Mar. 7		" " "

Strain "Be" Date of Inoculations- Jan. 31, 1930

B1	P*-2 hole	Mar. 14	Apr. 30	small,separate,flat,
B8	" " "	" "	Apr. 15	small,moist,heaped-up
B5	" 1 hole	" "	Apr. 21	small,heaped-up
B7	" " "	" 7	Mar. 21	contaminated by mold
B6	cotton plug-no P*	Mar. 14	Mar. 21	1. small,flat, separate 2. heaped-up,wrinkled

*Paraffin

TABLE 19

Strain "H37" Date of Inoculations- Jan. 31, 1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approximate date of Presence of Considerable Growth	Appearance of Growth
H1	cotton plug-no P*	Feb. 14	Mar. 21	distinct, wrinkled
H2	" " "	Mar. 7	Apr. 2	" "
H3	P-1 hole	Feb. 24	Mar. 21	" "
H4	" " "	" "	" "	" "
H5	" 2 "	" "	" "	slightly wrinkled
H6	" 2 "	" "	" "	button-like, wrinkled
H7	" 1 "	" "	" "	heaped-up, wrinkled, spreading
H8	" " "	" "	" "	" "
H9	" " "	" "	" "	" "

*Paraffin

Experiment 5

Potato Extract Glycerol Agar.

Media containing potato or potato extracts used for the cultivation of the tubercle bacillus are perhaps as well known and for some purposes more useful than glycerol agar. Two of the better known media in which potato plays an important part are those of Petragnani and of Petroff. Calmette and Guerin are using bile potato medium in the production of supposedly avirulent types of tubercle bacilli. Calmette, Boquet and Negre (1921) claim that bile-treated bacilli have been so modified, that if replanted upon ordinary potato-glycerin-broth, they assume the normal appearance of tubercle bacilli, but never regain virulence and remain incapable of producing tubercles.

In studying the nature of the growth-promoting principle in the potato, Uyei came to the conclusion that inositol maltose and dextrose (present in the potato) are metabolic stimulants, stimulating the growth of tubercle bacilli only when these are present in large numbers, whilst soluble starch and dextrin, but not glycogen are reproduction stimulants, effective when tubercle bacilli are present in any numbers.

Corper and Uyei (1929) suggest that whenever it is desired to take advantage of the growth-promoting properties of potato incorporated in a solid medium similar to an agar medium

for growing pure transplants of tubercle bacilli, that the following simple medium be considered.

Mashed autoclaved potato	25% by weight
Glycerol	2.5% " "
Agar-Agar	1.5% " "
Distilled H_2O	71% " "

Arloing and Dufourt found (abstr. A.R.T.)"that human tubercle bacilli from homogeneous liquid cultures grown on potato jelly assumed in twenty days remarkable filamentous shapes. At times these filaments were 15 or 20 times as long as normal bacilli, while again they formed a much subdivided mycelium. These filaments were only slightly acid-fast and did not take the gram stain well. Occasionally they appeared very granular. When transplanted to the usual media they again assumed normal shape and staining properties."

EXPERIMENTAL

Inoculations were made, using all seven strains of the tubercle bacillus, onto Medium E.

Table 20 gives the results obtained. It is evident that about six to ten weeks were required before growth occurred. Only "G624" did not present signs of growth at the end of ten weeks.

MEDIUM E

Potato Juice Agar

1. Five hundred grams of potatoes, autoclaved previously 30 minutes at 15# pressure, were sliced into small pieces unpared. To this was added five hundred cc. of distilled water. The mixture was shaken intermittently for one hour. Then 500 cc. of water were again added, the mixture filtered, and made up to 1000 cc.
2. To the above filtrate were added:-

   Glycerol 20g.

   Difco Bacto Nutrient Agar 10g.

   The mixture was now thoroughly dispersed with the aid of heat and filtered through cotton.
3. It was then tubed, autoclaved thirty minutes at 15# pressure, slanted, and cooled.
4. The pH of the meddium while hot (after autoclaving) was 6.2.

TABLE 20

Date of Inoculations-Feb. 6, 1930.

Tube No.	Type of stopper	Appearance of growth
T_{21}	P* - 1 hole	slight growth after 10 weeks.
H_{21}	" " "	" " " 6 "
B_{21}	" " "	" " " " "
Z_{21}	" " "	large growth over surface and siders in 8 days.
G_{21}	" " "	no growth in 10 weeks
D_{21}	" " "	slight growth after 6 weeks.
S_{21}	" " "	" " " " "

*Paraffin

EXPERIMENT 6

Glycerinated Potato

Glycerinated potato medium and its various modifications (such as the addition of dyestuffs) constitute some of the best means we have for the cultivation of the tubercle bacillus. It is perhaps in some respects superior to potato extract glycerol agar in the matter of promoting growth. Also in using cultures on glycerinated potato for inoculation purposes the growth can be scraped quite readily from the surface of the potato since the latter presents a firm foundation. An interesting observation of Arloing and Malaitre is that colonies of tubercle bacilli (human, bovine, avian and fish) on potato media do not penetrate the interior of the media. Between the surface of the potato and the inner surface of the colonies there is a nebulous zone, where there are no bacilli and which is entirely blue-staining. The bacilli assume a frankly acid-fast aspect only in the most superficial layer of the colonies.

Bosson and Baudy used glycerinated potato in isolating tubercle bacuilli from sputum with very satisfactory results. After preliminary treatment with 6% sulphuric acid particles of saliva were sown on glycerinated potato, and bouillon added carefully

EXPERIMENTAL

Inoculations were made, using all seven strains of tubercle bacillus, onto medium F.(Glycerinated Potato). All The cultures with the exception of G624 (See Table 21) gave satisfactory growth on this medium; initiation of growth occurring in about three to five weeks.

MEDIUM F

Glycerinated Potato

(A. D. Pawlowsky-Ann. de l'Inst. Past. 1888, 2, P.302)

1. Semi-cylindrical sections are cut out of large tubes by means of a punch. After the skin is removed from the ends each piece should be 5 to 6 cm. long and have a diameter corresponding to that of the tubes.
2. The freshly cut sections should be quickly immersed in a dish containing a one percent solution of sodium carbonate, where they are left to soak for one or two hours, then dried on a cloth and a single piece put in each test tube.
3. The test tubes should be constricted at the junction of the middle and lower thirds. These tubes should have been previously filled with broth, or 5% glycerinated physiological salt solution up to the constriction level.
4. The tubes are plugged with cotton and sterilized once in the autoclave for 30 minutes at 120 ^{o}C. They are then sealed with sterilized rubber caps to prevent evaporation.

Our Modification (Medium F.)

1. Whole potatoes were autoclaved thirty minutes at fifteen pounds pressure. They were then cored into the form of cylinders with the aid of a cork borer. From these cylinders slants were cut and distributed singly to test tubes.
2. To each tube a small amount of solution (containing 8.5g. of NaCl and 40g. glycerol in 1000 cc. water) was added.
3. The tubes were autoclaved for thirty minutes at 15# pressure and cooled.

Medium F.1

To each of four of the above tubes four drops of .015% crystal violet were added.

TABLE 21

Date of Inoculation-Feb. 5, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
T22	P*--1 hole	Mar. 14	slight growth	slight growth
B22	" "	Feb.25	Apr. 11	flat, white
Z22	" "	Feb. 10	Feb. 25	large spreading, orange , yellow
H22	" "	Feb. 22		slight growth
G22	" "	no growth		
D22	" "	Feb. 25	Mar. 14	slight growth
S22	" "	Feb. 14	Apr. 4	slight growth

EXPERIMENT 7

Gentian-violet Potato -Juice

The medium used in this experiment is very similar to that of experiment 5 with the exception that the latter lacks gentian violet. Since the cultures in both experiment 5 and 7 were inoculated on the same day it is possible to get some idea of the effect of the gentian violet on the growth of the organism.

The use of dyes in preparing media to be specifically used for cultivating tubercle bacillus is chiefly for the purpose of isolating the latter from contaminated materials, such as sputa, etc. Several dyes have found a prominent place in work dealing with the above aspect of the subject. Among these are malachite green, gentian violet and crystal violet. Nearly all the dyes used, if insufficient concentration, are also capable of inhibiting the growth of the tubercle bacillus, and therefore should be used with care. Corper and Uyei (1929) recently found that crystalviolet when incorporated in a medium of mashed potato and agar exerted a deleteriuos effect on the growth of the tubercle bacilli.

EXPERIMENTAL

Inoculations were made, using all seven strains of the tubercle bacillus, onto medium : (Gentian-Violet-Potato-Juice Agar.)

From a comparison of Table 22 with Table 20 it becomes apparant that gentian violet did not exert any very marked influence for against the growth of the various strains. This satisfactory result may perhaps be due to a more uniform distribution of the dye than occurs in the case of potato slants impregnated with the dye.

MEDIUM G

Gentian-Violet-Potato-Juice Agar

1. Five hundred grams of whole potatoes were broken into pieces about one inch in diameter, with the skin included.
2. The potato pieces were shaken intermittently for forty-five minutes with 500cc. of physiological normal saline. The mixture was then filtered through towelling.
3. The filtrate was made up to 1000 cc. with distilled water. To this was added 23 g. Bacto Nutrient Agar and the mixture was brought to a boil.
4. To this were added 25 g. glycerol and 20 cc. of .0015% crystal (gentian) violet solution in 1% sodium carbonate solution.
5. The boiling mixture was filtered and tubed. After autoclaving for thirty minutes at 15# pressure, the pH, (determined colorimetrically with brom thymol blue) was 6.4.
6. The tubes were then slanted and cooled.

TABLE 22

Date of Inoculations--Feb. 6, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
T23	P*--1 hole	no growth		
H23	" "	Mar. 7	Apr. 11	distinct, heaped up wrinkled.
Z23	" "	Feb. 14	Mar. 21	heaped up, wrinkled
H23	" "	Feb. 19		
G23	" "	no growth		
D23	" "	Mar. 14		slight growth
S23	" "	Feb. 25	Apr. 11	heaped up, wrinkled.

EXPERIMENT 8

Potato-egg glycerol Medium

Since potato media and egg media are so beneficial for the growth of the tubercle an attempt was made in this experiment to deterime the possibility of accentuating the usual qualities of each by combining the principal ingredients in one simple medium.

This attempt was somewhat along the lines of Petragnani's medium which contains potato and egg as well as other substances but is rather different to prpare. (See Exp. 11).

EXPERIMENTAL

Inoculations were made in petri dishes prepared as described under Medium H.

Uncontaminated growth occurred only in the case of H37. Gentian-violet did not appear to inhibit the growth of this strain. The colonies were numerous and located mostly on the portion of the then layer of corgulated egg which overlay the slice of potato. Growth was visible in about 12 days which is rather unusual when considered in the light of the length of time necessary for growth in the case of other media on which H37 was inoculated.

MEDIUM H

1. Three eggs were cleaned, opened carefully, and the yolks separated from the whites.
2. The yolks and the whites were then placed in separate beakers containing 200cc. of 3% glycerol soln and stirred.
3. Potatoes were then pared and sliced in $\frac{1}{2}$" slices. A slice slice was placed in a clean and sterile dish. Over the potato slice was poured about 10cc. of egg yolk glycerol soln.
4. Sterilization was obtained by autoclaving for 20 min. at 15# pressure.

Medium H.
(Our Modification.)

1. Equal volumes of egg yolk glycerol soln. and egg white glycerol soln. were mixed thoroughly.
2. Gentian-violet is added in sufficient amount to make a final proportion of 1;10,000.
3. Continue as in (3) Medium H.

TABLE 23

Date of Inoculation Apr. 29, 1930

Petri dish No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H415		May 10	May 15	numerous, scattered, knob-like, wrinkled colonies.
D415		no growth by June 4		

Glycerol-gentian-violet-potato-egg-Medium H.

H416		May 10	May 15	scattered, wrinkled, heaped up colonies.
S416		contaminated		
D416		no growth by June 4.		

Experiment 9

Experiments with Tissue Media

Since the natural habitat of the tubercle bacillus is in the body it can be readily seen that attempts at cultivation of the organism should include media which simulate to a certain extent at least, the environment in the body, such as animal tissues or media containing tissue extracts. Kowalski (1890), for example, found that a medium which contained lung infusion, gelatin, egg white, as well as added salts would favor the growth of the tubercle bacillus as well as other"delicate" organisms. Ficker (1900) utilized brain tissue and brain infusion since his attempts at cultivation with the use of lung, testicle, spleen, liver, pancreas, udder,, kidney and other organ tissues indicated the superiority of the brain as a nutritive medium. Similar results were obtained from the brain tissues of horses, sheep, calves, cows, and humans. In their interesting study , A. Lumiere and L. Lumiere (1906) were able to obtain very rapid growth on pieces of liver or spleen which after cooking for forty minutes and soaking in 6% glycerol water were cut in pieces and sterilized in tubes at 120°C. Their results were corroborated and elaborated upon by deVecchi (1906) who found that other organs than liver and spleen were suitable for cultivation, but in varying degree. His best growths occurred on lung,

kidney and spleen, less favorable results with brain, liver, or, thymus, while poor cultures were obtained through the use of heart, pancreas, lymph, gland, or muscle. Human organs were equally good when compared with cattle organs, while cannine organs yielded even better growth. Gioelli (1907) reported growth after four days, no matter whether the bacilli were from culture or tissue pulp, if the medium consisted of pieces of human placenta placed in 6% glycerin broth and sterilized in plates or tubes. von Szaboky (1907) claimed that colonies developed in one day if glycerin agar containing lung infusion, peptone and glucose were used, providing the reaction was between 0.5% alkaline-0.5% acid. A medium similar to the above, but containing 16% sputum, enabled growth of B. tuberculosis to be seen in two days. Siebert (1909) making use of a horse meat infusion together with peptone and glycerin, also reported favorable results. Frugoni (1910) recommended the use of fresh rabbit lung or other tissue which after soaking for one hour in 6% glycerine-in-physiological saline solution, was moistened by 6% glycerine bouillon and sterilized. Following the idea proposed by Gioelli, Wellmann (1912) was successful in growing strictly parasitic or feebly saprophytic bacteria including B. tuberculosis on a placenta infusion, sterilixed by Berkefeld filtration and heating to 40-41°C. for 2 d. to inactivate complement. Porter(1917) who determined the bacteriolytic actively of organ extracts on tuberculosis, found a consistent

relationship between lipolytic actively and tuberculolytic power. Accordingly pancreas extract exerted the most potent bactericidal effect, while the least effective was lung extract. Liver, thymus and lymphatic glands were strongly bactericidal. Other organs such as brain, thyroid, spleen, suprarenals, bone marrow, and pituitary glands were bactericidal to a lesser degree. One human skin extract which was fatty and cloudy in appearance as well as exceptionally rich in esterases proved to be extremely bactericidal. Other acid-fast bacilli were also bacteriolyzed by the above extracts although less susceptible than B. tuberculosis. Pig's lung extract which contained an unusually large amount of olein lipase and which had no effect on tubercle bacilli, destroyed these other acid-fast bacilli.

More recently, Moureau and Gruvel (1926) obtained good growth with extracts of lungs, spleen and liver, prepared by treatment of the tissues with glycerine-saline. Liver juice extract prepared in this way gave growth which was slower and less abundant than that obtained through the use of lungs and spleen. (The reason for this may be seen from the above) It was concluded, however, that such costly and somewhat complicated media did not seem to present any great advantage over the media ordinarily used.

Experiment 9

In order to determine the suitability of tissue preparations and tissue extracts for the growth of the tubercle bacillus the following experiment was performed. A bovine strain (1698) and a human strain (H37) were used. Inoculations were made from active growing cultures of the organisms into tubes containing the tissue extract or the pieces of tissue prepared as described in the section on media. The date of inoculations was May 11, 1930. To stopper the tubes only cotton plugs were used-no paraffin. The results obtained were uniform for all the media employed, i.e., no growth was visible in 15 weeks. For purposes of reference the media used and the tubes inoculated are given below:

Table 24.

a. Liver tissue	H450	H451	D450	D451
b. Lung tissue	H452	H453	D452	D453
c. Heart Muscle tissue	H454	H455	D454	D455
d. Spleen tissue	H456	H457	D456	D457
e. Veal infusion	H458	H459	D458	D459
f. Lung extract broth	H460	H461	D460	D461
g. Spleen Extract Broth	H462	H463	D462	D463
h. Heart muscle extract broth	H464	H465	D464	D465

These results, while apparently contradicting the results of previous workers, may be explained on the basis that the particular tissues used were unfavorable, although lung tissue and lung extracts have been used before with positive results.

MEDIUM I.

Tissue Media

(Lumiere, A. & Lumiere, L. --C. R. de Soc. de Biol. 1906, 60, p.568)

1. Pieces of liver or spleen from ox or calf are cooked for forty minutes in an autoclave.
2. They are then cut up into square prisms, washed with distilled water, and immersed for one hour in 6% Glycerol water.
3. They are finally tubed in potato tubes and sterilized 15 min. at 120 °C.

Our Modification (Medium I)

1. Fresh heart, spleen, liver, and lung (of calf) were used.
2. After autoclaving and washing, the prisms which had been cut were immersed in 6% glycerol solution and kept for six days in the refrigerator.
3. To each tube containing the tissue prism was added a small volume of the glycerol solution in which they had been soaked.
4. Autoclaving was at 17# pressure for 15 minutes.

Tissue Extracts (Our Modification)

1. The watery extracts obtained above by boiling the tissues with water for 40 minutes in the autoclave were placed in the refrigerator for 6 days. They were then tubed separately, 10cc. to each tube, and were autoclaved for 15 minutes at 15# pressure. They were then ready to be inoculated.

Experiment 10.

MEDIUM OF BESREDKA AND JUPILLE

One of the most interesting media for the cultivation of the tubercle bacillus is that of Besredka and Jupille (1913). In this medium, as Calmette notes, growth appears deep in the medium rather than at the surface only. Certain strains of tubercle bacillus show very definite signs of growth within the first few days after planting. Bronfenbrenner (1917) in his work on complement fixation in tuberculosis found this medium to be excellent for the cultivation of organisms to be used for antigen.

EXPERIMENTAL

To determine the value of this medium for the cultivation of the tubercle bacillus inoculations were made in triplicate into tubes containing fifteen cc. each, using one human (H37) and one bovine (1698) strain. The tubes were sealed with cotton plugs only paraffin being considered unnecessary. The relevant data will be found below. No growth was visible in any tube, even after fifteen weeks of incubation at 37° C.

Table 25

Strain	Tubes.		
H37	H470	H471	H472
	D470	D471	D472
1698			

MEDIUM J

Bearedka's Medium

(J.J.Bronfenbrenner, J.Lab. & Clin. Med. 1917, v, 3)

1. Each, white and yolk of egg are diluted with ten volumes of water and filtered through a hard paper (Chardin used by author).
2. The yolk solution is carefully clarified by the gradual addition of NaOH. Both solutions are autoclaved and kept separate.
3. Just before using, one mixes ten volumes of the sterile veal infusion (prepared without peptone, salt, or glycerol) with two volumes of the sterile egg white solution and one volume of the clarified egg yolk solution.
4. The mixture is placed in sterile tubes and used without further sterilization.
5. As this medium deteriorates on standing it should be made fresh eadh time.
6. Certain strains of the tubercle bacillus show very definite growth within the first few days after planting.

Our Modification (Medium J)

1. Instead of using hard Chardin filters we used the ordinary fluted filters used for biochemical work.
2. Both of the egg solutions, after autoclaving, were kept separately in the refrigerator for two weeks.
3. The sterile veal infusion was prepared by placing in the icebox the following:-

   1000 cc. of distilled water to which was added one pound of finely-cut veal.

   After infusing for seventy-two hours, the flask containing the material was heated in a water-bath at 45C for one hour, then heated in an autoclave(door open) with streaming steam for 30 minutes. It was then strained through clean towelling and placed in the refrigerator for four days.
4. Just before using, two volumes of the sterile egg white solution were mixed with one cc. of sterile egg yolk solution and to this was added ten cc. of veal infusion(sterilized an hour before for 25 minutes in the autoclave at 15# pressure) The above amounts were put in each of six tubes, which were then refrigerated for twenty-four hours before using.

Experiment 11.

Petragani's Medium

The medium of Petragnani has found considerable use, particularly among European investigators. This medium (Petragnani, 1926) which contains not only glycerol and eggs but also grated potato and potato meal, is further distinguished by the use of malachite green which is supposed to diminish over-growth by contaminating micro-organisms. Frongia (1927) has studied the effect of malachite green on pure cultures of tubercle bacilli; the presence of the dye in concentrations of 0.05-0.3% seems to favor the growth of the organism, although in higher concentration than this it inhibits the development of many strains. The malachite green also prevents the growth of molds. Sonnenschein (1928) advocates the use of Petragnani's medium for the isolation of tubercle bacilli from milk. Bang (1930) has found that Petragnani's medium, even without malachite green is practically equal to Lubenaus medium and far superior to that of Sweany and Evanoff. Malkam (1930) could find little difference in the relative values of the Petroff and Petragnani methods for isolation of the tubercle bacilli. Blechman (1927) on the other hand concluded from his study of these two media that the medium of Petragnani was to be preferred, it yielding more numerous positive results and these appearing more quickly. In confirmation of this Lichtenstein(1928) in a preliminary comparative study using the media of Petragnani, Petroff, Dorset, Lubenau, and Hohn found that

Petroff's medium was of little or no value, while that of Petragnani was distinctly superior to the others.

Experimental: Four tubes each of Petragnanis medium were inoculated with H37 (human strain) and 1698 (bovine strain) respectively. After inoculation at 37°C. the tubes were examined from time to time for signs of growth.

It will be seen from Table 26 that only one tube each of the two strains, showed signs of growth. Even here the growth was not very extensive. Two things are worth noticing in the above experiment. First, growth occurred using both human and bovine strains of bacilli, which would tend to conform with the recommendations of Sonnenschein (1928) since the tubercle bacilli found in milk are likely to be of bovine origin. With Petroff's medium and that of Corper and Uyei no growth occurred with bovine strains although these results are not conclusive since the number of tubes inoculated was small and the human strains themselves showed poor growth. Second, no contamination occurred in any of the tubes containing media which had inhibitory dyes, while tubes of other similar media which did not have inhibitory dyes added, were all to frequently contaminated and even liquified.

The results obtained here are not as clear cut as they might be. Subsequent investigation by one of us has shown that Petragnani's medium is excellent for the isolation and cultivation of tubercle baculli

from sputum, being equal to the medium of Corper and Uyei while a superior to the medium of Petroff.

Medium K.

Medium of Petragnani

(Lichtenstein, C. --Centr. f. Bakt. v.108 p.239)

1. Mix 150 cc. of milk, 6 grams of potato meal, and a piece of potato the size of an eff, chopped fine.
2. Place in a boiling waterbath and shake for ten minutes, then let stand in the water bath for one hour. Cool to 50 C. and add four whole eggs, one egg yolk, 12 cc.of glycerol, and 10 cc. of 2% malachite green solution.
3. Shake vigorously and filter through gauze.
4. Tube and sterilize at 80 C. for twenty minutes on three consecutive days.

Our Modification(Medium K)

1. We used 200 cc. of milk instead 150 cc.
2. We did not add the extra egg yolk called for in the directions.
3. We inspissated at 80 C. for one hour on three consecutive days, rather than for twenty minutes.

TABLE 26

Petragnani's Medium

Date of Inoculations-May 11, 1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approzimate Date of Presence of Considerable Growth	Appearance of Growth
H_{411}	P----2Hole	no growth by June 4		
H_{412}	" "	" " " " "		
H_{413}	" "	May 15		slight growth
H_{414}	" "	no growth by June 4		
D_{411}	" "	" " " "		
D_{412}	" "	May 15		slight growth
D_{413}	" "	no growth by June 4		
D414	" "	" " " "		

Experiment
12.

Petroff's Medium

(Gentian violet-egg infusion medium)

Since the publication by Petroff in 1915 of his method for the isolation of tubercle bacilli from sputum, the medium which bears his name has been used rather widely, particularly in this country. Due to its interesting composition, other workers have made studies of the medium and have subsquently used it with their own modifications. One of the first to publish on Petroff's method was Keilty (1915) who found the medium to be of value. The studies of Williams and Burdick(1916) seemed to them at least to underate the desirability of using dilute solutions of egg yolk and egg white and of incorporating agar. Corper, Fiala, and Kollen (1918) found that veal infusion was not absolutely essential to the medium and that equally satisfactory results were obtained by the substitution of beef extract, peptone, and sodium chloride in place of the veal infusion. Limousin (1921) used half the quantities advocated by Petroff, for preparing the veal infusion; he also insisted on the necessity for absolutely aseptic precautions throughout . Despeignes (1922) also used half of the quantities recommended by Petroff and sterilized the beef infusion by heating 8 days at 55-57° for one hour each day. Blechman (1927) in a series of comparative tests using the medium of Petragnani as well as that of Petroff, found the medium of Petragnani to be more favorable to the growth and a greater number of positive

results. Corper and Uyei(1928--1929) during the studies on their new medium, also conducted a comparative study on using Petroff's medium. They found that their method of isolation using H_2SO_4 was superior to the NaOH method, that their medium was more satisfactory than Petroff's and that the medium of Petroff actually exercised aslight inhibitory effect on tubercle bacilli, rendering it unfit for continued cultivation of the organizm. Malkani (1929) however in a comparative study involving the methods of Corper and Petroff found that the difference in results depended on the reagent used for isolation. With the use of NaOH, Petroff's medium yielded slightly better results than that of Corper while the use of H_2SO_4 was advantageous for Corper's medium. The fact that occasionally colonies appeared on Petroff's medium using the NaOH method, while under the same conditions no colonies were to be found in Corper's medium and vice versa, this author explained on the basis of S and R" strains, saying that one strain favored the alkaline conditions while the other did not. Later, however, the same author (Malkani 1930) after comparing the methods of Hohn (using Lubenaus medium) Petragnani and Petroff, found that there was no great difference in the values of the three methods used.

EXPERIMENTAL:

Inoculations in duplicate, using four different strains of tubercle bacilli, two bovine and two human, were made onto slants of Petroff's medium and incubated at 37°. They were observed from time to time for

evidences of growth.

It will be seen from Table 27 that Petroff's medium did not prove especially favorable to the growth of the tubercle bacillus. Three strains failed to show any growth at all after six weeks, while the inoculations made with H37 showed only slight growth at this time. It seems quite likely that the gentian violet which is used to inhibit spore-forming possible contaminants exercises a somewhat deleterious effect on the tubercle bacilli as well since the other constituents of the medium are known to be extremely favorable to the growth of this organism.

MEDIUM L

Petroff's Medium

(Petroff, S. A. - J. Exper. Med. 1915, 21, p.38)

1. An infusion is prepared from 500g. of beef or veal in 500 cc. of water containing 15% glycerol. The meat is left for 24 hours in a cool place to macerate, after which it is squeezed in a sterile press and the infusion collected in a sterile beaker.
2. The shells of several clean eggs are sterilized by a ten minute immersion in 70% alcohol. They are broken in a sterile beaker, the yolks and whites well mixed by shaking and filtered in a funnel through sterile gauze.
3. A one percent alcoholic solution of gentian violet is prepared.
4. For use, add one part of meat juice to two parts of egg mixture by volume. Add the gentian violet solution to make a final proportion of 1;10,000. Mix the three ingredients well, and tube and inspissate in an inclined position for three quarters of an hour on three successive days.

Note:-

Calmette in his book recommends the use of meat infusion and egg mixture in the ratio of part for part. He also believes that on the first day the inspissated slants should be kept at 85° C until completely hardened, but should be at 75° C. for one hour only on each of the two days. The gentian violet may also be added to the meat extract before mixing with the eggs, in the ratio of 1 cc. of 1% alcoholic extract of gentian violet to each 100 cc. of extract.

Our Modification (Medium L)

1. The meat was infused in the 25° F. electric refrigerator for 72 hours.
2. We used meat extract and egg mixture in the proportion of one part meat juice to 2 parts egg mixture.
3. The tubes were inspissated as follows:-

1st day---2 hours at 90° C
2nd "----1 hour at 82° C
3rd "----1 hour at 82° C.

TABLE 27

Petroff's Medium

(Gentian-Violet Egg)

Date of Inoculation-April 29, 1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approximate Date of Presence of Considerable Growth	Appearance of Growth.
H_{406}	P*----2 hole	May 15		slight growth
H_{407}	" "	" "		" "
T_{405}	" "	no growth by June 4		
T_{406}	" "	" " " " "		
S_{404}	" "	" " " " "		
S_{405}	" "	" " " " "		
D_{404}	" "	" " " " "		
D_{405}	" "	" " " " "		

* Paraffin

EXPERIMENT 13

Medium of Corper and Uyei

In 1927 appeared the first published reports on the new medium of Corper and Uyei. These authors having found the medium of Petroff unsatisfactory and having noted the value of potato media for cultivation of tubercle bacilli as reported by earlier workers, decided to incorporate the advantages of the two. The choice was a happy one. The experimental results which they obtained led them to the conclusion that their medium was considerably superior to that of Petroff and that the use of sulphuric acid for the preliminary treatment in destroying contaminants was to be preferred to Petroff's sodium hydroxide method. Malkani (1929and 1930) found that the superiority claimed for the new medium was not so great; also, that the reagent used to treat the sputum was of significance in determining the presence or absence of growth on cultures. Corper's claims that the new method was superior to the older methods of diagnosis such as guinea pig inoculation and the examination of stained smears, were borne out to some extent by the results of Cunningham and Cummings (1930). These investigators found that the culture method was at least the equal of the smear examination in total number of positives obtained. They found furthermore, that a number of positives were obtained on culture which were negative on the examination of smears. They concluded that conatmination of cultures was avoided, the method of diagnosis by culture was a valuable aid and should be used in laboratories.

EXPERIMENTAL

Three tubes of Corper-Uyei medium were inoculated with H37, two tubes with Be and S1800 respectively, and after being incubated at 37° were observed from time to time for signs of growth.

It will be seen from the table which follows (Table 28) that the two strains Be and S1800 failed to show any growth whatsoever on this medium, even after six weeks. One of the tubes inoculated with H37 showed slight growth in three weeks, which did not increase very greatly in size however, even after six weeks.

Here, too, then apparently the gentian violet present is sufficient to restrain the growth of laboratory cultures of the tubercle bacillus.

MEDIUM M

Corper's Medium

(Corper & Uyei--J. Lab. & Clin. Med. 1928, 13,p.476)

1. Large clean potatoes free from surface defects are cut into cylinders about three inches in length and five inches in diameter, using for this purpose a cork borer. They are then halved longitudinally.
2. As soon as they are cut these potato cylinders are soaked in one percent sodium carbonate solution containing freshly added gentian violet in concentration of 0.003%.
3. After soaking from one to two hours, they are gently wiped with a clean towel to free them from excess of fluid and are then introduced into a sterile culture tube ($6x\frac{3}{4}$) containing $1\frac{1}{2}$ cc. of 5% glycerol bouillon.
4. They are plugged with cotton and then sterilized in an autoclave at 15# pressure for at least thirty minutes, after which they are ready for use.

Our Modification (Medium M)

1. We placed in each tube to which the potato slant had been added 1 cc. of a 6% aqueous solution of glycerol.
2. We halved the potato cylinders diagonally, giving us two slants from each cylinder.
3. We soaked the slants in the gentian violet solution for thirty minutes instead of two hours.

TABLE 28

Corper's Medium

(Crystal-Violet potato glycerol water)

Date of Inoculations-April 29,1930

Tube No.	Type of Stopper	Date of first Appearance of Growth	Approximate date of presence of Considerable Growth	Appearance of Growth
H_{408}	P*--2 hole	May 15		slight growth
H_{409}	" "	no growth by June 4		
H_{410}	" "	" "	" "	
B_{408}	" "	" "	" "	
B_{409}	" "	" "	" "	
S_{408}	" "	" "	" "	
S409	" "	" "	" "	

*Paraffin

EXPERIMENT 14

Dorset's Medium

The use of slants made of coagulated whole eggs for the cultivation of the tubercle bacillus has been thoroughly tested since the publication of the original paper by M. Dorset (1902). The use of such a medium for transplanting stock cultures of tubercle bacilli is very satisfactory and has found wide use in many laboratories, particularly in this country, being recommended in most of the standard texts. The medium which is made of well-mixed whole eggs to which a small volume of distilled water is added, is simple, easily prepared, and has been adopted with minor modifications in Europe as well. Abel(1912) recommends physiological salt solution to be added to the eggs. Brown and Smith (1910) use 24% distilled water rather than the 10% recommended by Dorset. Soparka (1916) adds 6.0 cc. of water containing 5% glycerol for each egg (72 cc. volume). Lubenau (1907) has modified the medium through the addition of glycerol bouillon. Hohn (1929) claims to have improved upon this latter medium through the addition of 2% Haematin (hemoglobin solution).

The medium has also been utilized for the isolation and cultivation of tubercle bacilli from tuberculous material. Lichtenstein (1928) found it to be superior to the medium of Petroff, but inferior to that of Petragnani. Harada (1929) found that Dorset's medium was quite satisfactory and when 0.05% malachite green had been added to diminish contamination, it was not inferior to the Petragnani medium.

Experimental

(a) Dorset's medium - without glycerol.

Inoculations were made onto tubes of Dorset's medium using two human strains (H37 and S1800) and two bovine strains (T273 and 1698). The cultures were incubated at 37°C.

It will be seen from Table 29 that this medium did not serve as a favorable medium for the continued growth of our strains of the tubercle bacillus. Considerable trouble was experienced here, as elsewhere from mold contamination, but eliminating these, it will be seen that in none of the tubes of each strain which remained uncontaminated was there any growth.

(b) Dorset's medium + 3% glycerol

Inoculations were made in duplicate onto tubes of our modification of Dorset's medium containing 3% glycerol, using the strains mentioned above.

It is evident from Table 30 that contamination occurred frequently in this series as well, but the tubes which remained uncontaminated showed more favorable results. Thus one tube each of both human strains showed a slight but definite growth as denoted by an increase in size, as did one tube of the bovine strain 1698. This is in partial conformity with the findings of many workers who report that the human strain grows best on media containing glycerol, while the bovine strain grows better on media lacking glycerol. Perhaps the single tube containing

bovine organisms showed growth which took place in spite of , rather than because of, the glycerol. Furthermore this strain has shown growth on ösheä media containing glycerol, so that it may be regarded as a strain of bovine origin which by development on artificial media has become adapted to its environment to the extent of being able to utilize the glycerol.

MEDIUM NY

Dorset's Egg. Medium

(Dorset, M. -- Amer. Med. 1902, 3, p.555)

1. Hens eggs are carefully scrubbed with a brush in boiled water and then immersed for some minutes in a 5% carbolic acid solution.
2. They are next taken between two sheets of sterile blotting paper, the two ends flamed and a hole made in each with pointed flamed forceps.
3. By means of a rubber tube containing sterile cotton as a filter, the contents of the eggs are blown into a previously weighed sterile Erlenmeyer flask. The egg should be blown from the upper or air chamber end.
4. A quantity of water equal to 10% of the weight of the egg is next added to the flask, which is shaken to assure a homogeneous mixture of the yolk and white and the water, care being taken to avoid air bubbles.
5. The whole is then put through sterile heavy muslin in a funnel and the filtered mass portioned out a aseptically in test tubes.
6. The tubes are coagulated by being put in a thermostat in an inclined position, at 70 ^{o}C. for two hours.

They are then left in the incubator at 37° C. for 3 days in order to detect any contamination. Finally they are sealed and kept in a vertical position until used.

Our Modification (Medium $_N$)

1. The eggs were thoroughly washed, then immersed in 70% alcohol for 10 minutes.
2. The eggs were broken into a beaker and for each 100 cc. of egg, 20 cc. of water was added. The egg and water were then mixed as thoroughly as possible, at the same time avoiding the production of air bubbles.
3. The mixture was strained through sterile cheesecloth, tubed in 10 cc. portions, and coagulated in a slanted position.
4. The tubes were kept at 90° C. for 2 hours the first day to bring about coagulation. They were then put in the incubator at 37° C. for 24 hours to detect contamination; the second day they were heated in an electric oven at 82° C. for 1 hour and incubated at 37° C. for 24 hours; on the third day they were again heated at 82° C. for 1 hour and then incubated at 37° C. for 24 hours.

Glycerinated Egg Slants

(Our Modification)

Dorset's egg medium (modified) was prepared as above, except that for each 100 cc. of egg volume there was add 3 cc. of glycerol. The filtered, tubed mixture was inspissated as above to bring about sterilization.

TABLE 29

Dorset's Medium

(no glycerin)

Date of Inoculation April 29, 1930

Tube No.	Type of Stopper	Date of first Appearance of growth	Approximate date of presence of considerable growth	Appearance of growth
H_{400}	P*-2 hole		contaminated	
H_{401}	" "		"	
H_{402}	" "		no growth by June 4	
T_{400}	" "		contaminated	
T_{401}	" "		"	
T_{402}	" "		no growth by June 4	
S_{400}	" "		contaminated	
S_{401}	" "		no growth by June 4	
D_{400}	" "		contaminated	
D_{401}	" "		no growth by June 4	

*Paraffin

TABLE 30

Dorset's Medium & 3% Glycerin

Date of Inoculation April 29, 1930

Tube No.	Type of Stopper	Date of first appearance of growth	Approximate date of presence of considerable growth	Appearance of growth
H_{403}	P*-2hole		contaminated	
H_{404}	" "		"	
H_{405}	" "	May 15		slight growth
T_{403}	" "		contaminated	
T_{404}	" "		"	
S_{402}	" "		"	
S_{403}	" "	May 15		slight growth
D_{402}	" "		contaminated	
D_{403}	" "	May 15		slight growth

*Paraffin

Experiment 15

The following experiment, which is in reality a series of experiments, involves the use of various media which have as a common base 3% glycerol agar. To this base various substances (See medium T) were added to give a consentration of 1%. Although the substances vary greatly they are all included here in order to simplify the presentation.

MEDIUM O

Glycerol Agar plus Additional Substances

1. A 3% glycerol agar medium was prepared by mixing the following:-

Bacto Nutrient Agar	23.0 g.
NaCl	8.0 g.
H_2O (distilled)	1000.0 cc.
Glycerol	30.0 g.

2. The mixture was brought to a boil and filtered. It was then tubed, 9 cc. per tube, and to each tube was added a 10% mixture of various substances (given below). The agar and added mixture were thoroughly shaken, and then autoclaved at 15# pressure for 20 minutes, slanted and allowed to cool.
3. The mixtures were prepared by weighing out 1 gram of the substance (see below) and adding 9 cc. of H_2O, and thoroughly shaking the tubes to suspend the materials in the water. When the materials were suspended, 1 cc. of suspension was pipetted from the original tube into the tubes containing glycerol agar. This gave a 1:100 dilution or a 1% concentration of the substance in the agar.
4. 10% suspensions in water of the following compounds were prepared:-

a. gelatin
b. orcinol
c. vanillin
d. asparagin
e. creatinine & creatine
f. leucine
g. hemoglobin
h. cholesterin
i. sodium glycerophosphate
j. sodium taurocholate
k. calcium saccharate
l. atroping sulphate
m. brucine
n. casein
o. egg albumin
p. pepsin
q. pancreatin
r. glycocoll
s. alloxanthin
t. oxgall
u. sodium sulphindigotate
v. sodium benzene sulphonate

Three tubes of each compound were made.

Experiment 15a.

This experiment was carried out to determine the effect on the growth of the tubercle bacillus of certain amino acids and closely related compounds.

Glycine

The value of the more common amino acids for cultivation of the tubercle bacillus has been more or less thoroughly investigated by workers since the isolation of the organism. Proskauer and Beck (1894) found that glycine was of distinct value in the growth of the organism although not as good as a combination of amino acids such as leucine and tyrosine. Armand-Delille, et al (1913) obtained very good growth in a synthetic medium containing not only salts, glucose and glycine, but also glycocoll and arginnine. Wherry (1914) found it to be slightly favorable to growth as did Long (1924- 1925). Kardo (1924-1925) found that when amino acids alone formed the only source of both nitrogen and carbon, the organisms yielded little or no growth. If oalate was used as a second source of carbon, growth took place in media containing glycine, but better with alanin, leucine, or asparagin, although there was considerable variation in this respect among the different strains employed. It will be seen from Table 31 that good growth resulted when glycine was added to the medium. The results of previous investigators are thus borne out in this case.

Leucine

In their comprehensive study Proskaur and Beck determined that leucine was favorable in promoting the growth of the organism under study. Even better results were obtained when it was reinforced by another amino-acid such as alanine or tyrosine. According to Wherry (1914) leucine was better than glycine, while superior to both was alanine. Similar results were obtained by Long (1922). Kondo (1925) also reported the superiority of leucine over glycine and tyrosine.

EXPERIMENTAL

Three tubes of leucine glycerol agar were inoculated with H37 and after the usual sealing procedure were inubated at 37oC. After 4 weeks there appeared small isolated pinkish wrinkled colonies, which reached their maximum development in six weeks. These results, which indicate that the leucine used was not particularly favorable to growth, contradict the results obtained by the other investigators.

Asparagin

The value of asparagin in promoting growth of tubercle bacilli in media which contain it, has long been know. Proskauer and Beck (1894) working with Kuhne's medium found that asparagin was desirable for growth promotion; it if was missing, but leucine were absent, even though asparagin were present, the growth was diminished. Asparagin was held to be desirable for growth even as were leucine and alanine. Mieke (1908) included asparagin

in his basal solution when he wished to study carbon sources suitable for B. tuberculosis. Kendall, Day and Walker (1914) also utilized asparagin, realizing its desirability in culture media, as did Calmette, Massol and Breton(1909) and Wherry (1914). Lowenstein (1913) in his basal solution included asparagin and more recently Lowenstein (1927) has introduced this compound into an egg medium which is used for the isolation of the tubercle bacillus. Besancon (1920) recommends the use of asparagin, as in Frouin's glycerol asparagin solution. Goris (1920) in his studies on the acid-fastness and chemical composition of the tubercle bacillus used a solution containing asparagin, as did Borrel, de Coulon, Boez and Quimaud (1922). The medium of Long (1925) which has been used in growing cultures for the determination of the chemical composition of the tubercle bacillus makes use of the growth-promoting qualities of asparagin. Henley (1930) while admitting the value of asparagin, attempted to find a substitute for it in the form of ammonium malate, because of the expense of asparagin.

EXPERIMENTAL

Inoculations in triplicate using H37 were made into tubes of 1% asparagin- 3% glycerol agar. After being paraffin-2hole sealed, these tubes were incubated at 37°C. As shown in Table 31 good growth was obtained in 4-6 weeks. The colonies were heaped up and wrinkled and somewhat larger than those in the 3% glycerol

agar control tubes. These experiments indicate that the asparagin has a somewhat beneficial value, confirming the results of earlier workers.

TABLE 31

Compound -- glycine — Date of Inoculations Apr. 11, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of considerable growth.	Appearance of growth
H_{292}	P* - 2 hole	May 7	May 15	wrinkled, heaped up
H_{293}	" "	"	"	" "
H_{294}	" "	"	"	" "
Compound--leucine				
H_{248}	" "	"	"	small isolated colonies
H_{249}	" "	"	"	" "
H_{250}	" "	"	"	" "
Compound--asparagin				
H_{243}	" "	"	"	heaped up, wrinkled.
H_{244}	" "	"	"	" "
H_{245}	" "	"	"	" "

Experiment 15b.

Bile and Bile Salts.

Several investigators have noted the unusual effect of bile or bile salts on the growth and characteristics of the tubercle bacillus. Calmette and Guerin (1908) using their glycerin-bile potato were able to cultivate a strain of tubercle bacillus which was apparently entirely non-virulent and which has been used for prophylactic immunization under the name B.C.G. Larson and Montauk (1923) believed that the diminution in virulence was caused by the bile which lowered the surface tension and thus increased the "wetting" of the organisms rendering them more permeable and therefore more easily subject to the action of the antibodies and phagocytes of the host. Model (1929) in his work with capillary-active substances which decreased surface tension, could inhibit the growth of the organism if the tension were diminished below 0.700. In order to check these results the following experiments were prepared using whole bile (dehydrated) and sodium taurocholate, a bile salt.

EXPERIMENTAL

(a) Whole Bile.

One percent whole bile bovine was added to glycerin agar, three tubes of this medium were inoculated with H37 and incubated at 37 °C. It will be seen in Table 32 that

the bile in concentration of 1.100 had a distinct inhibitory effect upon the organism, completely inhibiting growth. This not only agrees with the results of other workers, but is also presumptive evidence in favor of Calmette's claim that bile may be used to distinguish bovine from human strains of tubercle bacilli; the basis for this being that human strains will grow in human bile while bovine strains are inhibited; similarly bovine strains can grow in bovine.

(b) Sodium taurocholate.

The following results were obtained through the use of 1% sodium tuarocholate-glycerin agar, inoculated with H37 and incubated at 37°C. The sodium tuarocholate had somewhat of an inhibitory effect on the tubercle bacillus, since growth was not as prolific as in the control glycerin-agar tubes. Inhibition was not complete, however and it seems probable that the substance or substances which enable the differentiation of human and bovine strains to be made in bile media, are not identical with sodium taurocholate.

TABLE 32

Compound-Na taurocholate Date of Inoculations Apr. 11, 1930

Tube No.	Type of stopper	Date of forst Appear-ance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H_{257}	P*--2 hole	May 15		slight growth
H_{258}	" "	"		" "
H_{259}	" "	"		" "
Compound--Oxgall				
H_{280}	" "	no growth		
H_{281}	" "	" "		
H_{282}	" "	" "		

Experiment 15c.

Cholesterol

The influence of cholesterol on the growth of the tubercle bacillus presents certain interesting features. On the one hand, Shape (1928) claimed that cholesterol prolonged the life of guinea pigs inoculated with virulent human type tubercle bacilli. On the other hand Model (1929) found that cholesterin favored the growth of the tubercle bacillus, while Lominski (1930) added lecithin and cholesterin to glycerin potato to favor the growth of the organism.

Some apparently contradictory results may be explained however. The value of the cholesterol in prolonging the life of guinea pigs experimentaily infected is probably due not to the cholesterol itself, but to the associated ergosterol (from which it is almost impossible to free cholesterol). Ergosterol being closely connected with vitamin D. may influence the animals to maintain their health and so resist the disease. More difficult is the problem of reconciling the results of Model and Lominski. Model determinations of the capillary activity of both lecithen and cholesterin have shown him that the two have a mutually antagonistic effect. Lecithin, which lowers the surface tension of the medium due to its capillary activity, inhibits growth, cholesterin which is very nearly insoluble, has no capillary activity and tends to favor growth. If both

are present, the cholesterin tends to absorb the lecithin and so favors growth. This antagonism is also shown in hemolysis. Why Lominski should add lecithen to the potato, therefore, to us, at least, somewhat uncertain.

EXPERIMENTAL

Three tubes of Glycerin agar to which a 1% cholesterol suspension had been added, were inoculated with H37, sealed with paraffin and incubated. It will be seen from Table 33 that the cholesterin present, slight though its solubility might be, still exercised a very considerable degree of favorable effect on the growth of the culture. This is in accordance with the results of both Model and Lominski.

Pancreatin

The enzyme pancreatin has been reported in the literature as having a strongly bactericidal effect on the tubercle bacillius. Porter (1917) found it to be the most strongly bactericidal of all the tissue extracts. Giese (1921) reported diminution of virulence of tubercle bacilli when exposed to pancreas tissue, while Day and Gibbs (1930) reported that the bacilli were killed by pancreatic juice but that there seemed to be no dissolution of the cell bodies or loss of acid fastness. The effect of "pancreatin" or the substances associated with this enzyme after inactivation by autoclaving, on the growth of B. tuberculosis was studied here.

EXPERIMENTAL

Three tubes were prepared by adding 1% commercial pancreatin to glycerol agar, then autoclaving and slanting. These were inoculated with H37 and incubated at 37°C. Excellent growth was obtained in these tubes as well be seen from Table 33. This, of course is not due to the pancreatin, which was inactivated by the heating, but probably to the substances with which the enzyme was associated.

Pepsin

Pepsin has not been reported as having any appreciable bactericidal powers when applied to tubercle bacilli; the action of tissue and gland extracts in destroying these bacteria has been ascribed to their lipolytic ferments rather than to any proteolytic powers they may contain. The effect of "pepsin" protein was here determined.

EXPERIMENTAL

Three tubes of glycerin agar were melted and to them added one percent pepsin; after sterilization in the autoclave (resulting in inactivation of the enzyme) the tubes were slanted and when from inoculated with H37 after incubation at 37°C. It appears that the substances with which the proteolytic enzyme is associated excerise no deleterious effect and under the conditions recorded above Table 33, actually favorable to growth of the organism.

TABLE 33.

Compound--cholesterol Date of Inoculations Aprl 11, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H_{251}	P*--2 hole	May 1	May 15	solft luxuriant, moist, wrinkled.
H_{262}	" "	"	"	" "
H_{253}	" "	"	"	" "
Compound--pancreatin				
H_{289}	" "	May 4	May 15	considerable, heaped up, wrinkled.
H_{290}	" "	"	"	" "
H_{291}	" "	"	"	" "
Compound--Pepsin				
H_{286}	" "	"	"	" "
H_{287}	" "	"	"	" "
H_{288}	" "	"	"	" "

Experiment 15d.

Creatine and Creatinine

Tuberculosis of the kidney is not infrequent. In this organ where creatin and creatinine are constantly being excreted what is the effect of these substances upon the invading agent? Armand-Delille et al (1913) include creatine in a medium which they say has the greatest advantages in growth of the tubercle bacillus.

EXPERIMENTAL

(a) Creatine

One tube containing 1% creatine added to glycerin agar, was inoculated with H37. Incubation at 37°C gave the result:- The creatine exercises no unfavorable effect upon the organism and actually seems to aid growth, since the colonies were large and flourishing.

(b) Creatinine

One tube of 1% creatinine-glycerine agar was likewise inoculated with H37 and incubated . As seen in Table 34, this compound also appears to be favorable to the cultivation and growth of the organism.

TABLE 34

Compound--Creatin		Date of Inoculations		
Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H_{246}	P*--2 hole	May 1	May 15	considerable, wrinkled heaped up
Compound--creatinin				
H_{247}	"	"	"	"

Experiment 15 e.

Proteins

The tubercle bacillus is often found in tissues where there are only very small concentrations of protein degradeation products such as peptones and amino acids, compared with the intestine. Furthermore, many investigators have grown the tubercle bacillus on media which contain proteins of various types such as tissues, tissue extracts, egg media, gelatin, casein, hematin, etc., It is often claimed, on the other hand that most bacteria are unable to attack pure proteins unless certian other substances are presnet to aid the organisms in their struggle to wrest food from these complex compounds. Just how valuable these proteins are we have tried to determine here in a preliminary fashion, altho much careful work is necessary with the aid of pure products before the problem can be satisfactoryily answered.

Egg Albumin

The value of egg albumin in growing the tubercle bacillus has been proven many times through the use of media containing egg yolk and whole eggs. Deycke (1895) made use of a glycerol agar containing alkali albuminate (from veal) with good results. Bezancon and Griffon (1903) used egg yolk glycerin agar. The numbers of workers who have used whole eggs is legion. Herrold (1951) used egg yolk again having left out even glycerol. He found this medium to be excellent for cultivation of tubercle bacilli from sputum. He recommended the omission of egg white from egg media since it seemed to be unfavorable to growth of the tubercle bacillus as well as other organisms. This is not surprising since it has been determined that egg white contains a substance, lyzozyme which has general bactericidal properties.

EXPERIMENTAL

1% egg albumin was added to glycerin agar, which was inoculated with H37. Incubation at 37°C. for 6 weeks gave the results recorded in Table 35. The egg albumin serves as a distinct source of nutrient material for the growth of the tubercle bacillus.

Casein

Marpmann (1903) has utilized a flour casein agar which helped obtain rapid growth of the organism which we are here studying. Likewise Valleti (1913) utilized a whey agar for the very rapid development of the tubercle bacillus.

EXPERIMENTAL

One per cent casein was added to glycerin agar. This medium was inoculated with H37 and incubated. The cultures showed good growth, Table 35, but not much superior to that obtained with the control 3 per cent glycerin agar tubes apparently the casein is not readily attacked by the organism under the conditions of this experiment.

Gelatin

Gelatin, which has been widely used in general bacteriology has been utilized by investigators in the field of tuberculosis as well. Matsuschits (1899) made use of gelatin media in his study of the differences in growth cultures avian and mammalian types of B. tuberculosis. Klimmer (1923) in his textbook gives the formula for a glycerol gelatin medium which is said to be satisfactory.

EXPERIMENTAL

Three tubes of gelatin glycerol agar were inoculated with H37 and after sealing were incubated at 37°C. The organism grows well on the above medium (Table 35) but the type of growth is different from most cultures; the colonies are dry and darker than usual. Apparently the protein has modified the cell chemisty of the organism, resulting in the changed appearance.

Hemoglobin

Lowenstein (1927) Dreyer and Vollum (1931) and a number of others have found that tubercle bacilli are almost always present in the blood-stream of experimental animals and humans ill with the disease. Do the organism in the blood stream take their nourishment from the surrounding fluid? Can they utilize complex proteins such as hemoglobin in their nutrituion?

Hohn (1929) believes they can. He has found that 2% hemotin added to Lubenaus medium favors growth, altho 3% hemotin is not so favorable. In order to check this point the following experiment was carried out;-

EXPERIMENTAL

One percent suspension of hemoglobin was added to glycerin agar and the whole autoclaved. The hemoglobin formed a solid button at the surface of the agar. Inoculations of H37 were made onto the agar surface, also directly onto the hemoglobin button, Incubation was at 37°C.

Results:-

Two of the three tubes so inoculated showed good growth One interesting feature was the observation that colonies of tubercle bacilli were found to be growing directly on the knobs of hemoglobin, even where they were not in contact with the surface of the agar. Apparently the organisms are able to use the hemoglobin in their metabolism.

TABLE 35

Compound--egg albumin Date of Inoculation Apr. 11, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Consideralbe growth	Appearance of growth
H_{275}	P* - 2 hole	May 1	May 15	wrinkled heaped up
H_{276}	" "	"	"	" "
H_{277}	" "	"	contaminated	
Compound--Casein				
H_{278}	" "	May 7	May 15	distinct heaped up wrinkled
H_{279}	" "	"	contaminated.	
Compound--gelatin				
H297	" "	May 1	May 15	dry, heaped up wrinkled.
H_{298}	" "	"	"	" "
H_{299}	" "	"	"	" "

Experiment 15 f.

Sodium Glycerophosphate

Buadran (1910) proposed the use of a glycerophosphate medium for the successful cultivation of B. tuberculosis, based on the theory that many of the most important compounds in the body contain the glycerinphosphate linkage and in the hope that from this glycerophasphate the tubercle bacillus would be able to synthesize compounds necessary to its growth. His medium included the glycerophosphates of sodium, calcium, magnesium and potassium in definite quantities, together with glycerin, albumose and other nutrients. This medium was so desirable that growth would take place even if glycerin were omitted, providing the sodium glycerophosphate concentration were increased.

EXPERIMENTAL

As usual three tubes of sodium glycerophosphate glycerin agar were inoculated with H37, sealed and incubated. The results after six weeks incubation may be seen in Table 36. Growth was quite considerable and the glycerophosphate did indeed seem to aid the organism, as Baudrau contended.

Calcuim saccharate

Although the tubercle bacillus apparently cannot utilize sucrose in its metabolism, it seemed worthwhile to see whether the substitution of a salt of this compound such as calcium saccharate, might not have a beneficial effect on growth.

EXPERIMENTAL

Three tubes of calcuim saccharate-glycerin agar were inoculated with H37 sealed with paraffin and incubated. After six weeks at 37°C. the results, recorded in Table 36, were observed. It will be noted from the above that the calcium saccharate had an inhibitiory effect on the growth of the organism, probably having some sparing action of the glycerin metabolism. This inhibition of growth was in contrast to the control tubes of glycerin agar which showed good growth at the end of the same period.

TABLE 36

Compound---Na glycerophosphate Date of Inoculations Apr. 11, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H_{254}	P*--2 hole	May 7	May 15	distinct, wrinkled
H_{255}	" "	"	"	" "
H_{256}	" "	"	"	" "
Compound--Ca saccharate				
H_{260}	" "	May 15		slight growth
H_{261}	" "	"		" "
H_{262}	" "	"		" "

Experiment 15 g.

Valnillin

The effect of vanillin, a natural product and one which finds considerable use in homes, on the continued devepoment of the organism should be interesting.

EXPERIMENTAL

Three tubes of 1% vanillin-glycerin agar were inoculated with H37 sealed and placed in the 37°C incubator. After six weeks the results recorded in Table 37 were noted:

Vanillin in the concentration used therefore appears to exhibit a decided inhibitory effect nullifying the beneficial properties of the glycerin.

Orcinal

Lewis (1917) found that a concentration of orcein of 1:1000,000 was the greatest which would permit growth of the organism. It was desired to check these results and to observe the effect of this benzene derivated on growth, so the following experiment was prepared.

EXPERIMENTAL

Three tubes of glycerin agar containing 1% orcinal were inoculated with H37 and incubated at 37°C after suitable sealing with paraffin.

From Table 37 it will be seen that the orcinal completely inhibited the growth of the organism, confirming the earlier work of Lewis.

Sodium Benzene Sulphonate

This benzene derivative which is more soluble in aqueous solutions then benzene, due to the sulfonation and subsequent substitutions with sodium, should indicate some of the properties of substances such as benzene. For this reason the following was performed:-

EXPERIMENTAL

Three tubes of 1% sodium benzene sulfonate glycerin agar were prepared and inoculated with H37. They were set aside in the 37°C incubator and observed from time to time. The sodium benzene sulfonate has a slight inhibitory effect (Table 37) since the growth was less then that with the control tubes containing glycerin agar. However, some slight growth did occur as shown by the small isolated colonies.

Sodium sulfindigotate

Another dompound which should be interesting is the above since it is a derivation of indigo whose solubility is increased by the sulfonation which has taken place and the presence of the eodium. To determine its effect, the experiment below was carried out.

EXPERIMENTAL

Three tubes of glycerin agar to which 1 percent of sodium sulphindigotate had been added were inoculated with H37 and incubated at 37°. The medium was green at the beginning of the experiment. In the two cases (Table 37) where the sulgindigotate had been converted to the colorless or leuco-form growth was not interfered with, as attested by comparison of the tubes with the control. One tube which remained greenish showing that the compound had not been completely changed to the colorless form, showed inhibition of growth. Since the change of color is probably due to oxidation or reduction it will be seen from the above that whereas a compound in one state of oxidation may influence growth of the tubercle bacillus in one way, in a different state of oxidation the effect may be the opposite. It is probable that similar results would be obtained with other organisms. It would be extremely interesting to make a study of a large number of com-

pounds in various states of oxidation or reduction and their effect upon bacterial metabolism; such a study would be of considerable significance in explaining the facts of metabolism.

TABLE 37

Vanillin — Date of Inoculation Apr. 11, 1930.

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H234	P*--2 hole	no growth		
H235	" "	"		
H236	" "	"		
Compound--Orcinol				
H231	" "	"		
H232	" "	"		
H233	" "	"		
Compound--Na benzene sulphonate				
H272	" "	May 22		small isolated colonies
H273	" "	"		" " "
H274	" "	"		" " "
Compound--Na sulfindigotate				
H269	" "	May 7	May 15	decolorized, distinct
H270	" "	"	"	" "
H271	" "	"	"	" "

Experiment 15H.

Alkaloids

The effect of alkaloids on the various tissues of higher forms of life has been studied quite extensively. The effect of these compounds on the microscopic forms however, has not been nearly as extensive altho the results should be equally interesting

A. Brucine

EXPERIMENTAL

1% brucine was added to each of three tubes of glycerin agar, which were then inoculated with H37 and incubated at 37°C After 15 weekd at this temperature the results recorded in Table 38 were noted.

Brucine in a concentration of 1:100 completely inhibits the growth of the tubercle bacillus, as shown above.

B. Atropine sulfate

Using the same methods and technique three tubes of glycerin agar containing 1% atropine sulfate were inocul ated with H37 and incubated as may be seen in Table 38. results similar to those with brucine were obtained. It appears from the above that the presence of alkaloids in concentration of

1:100 exerts a markedly deleterious effect on the growth of the tubercle bacillus.

TABLE 38

Compound--Brucine Date of Inoculations Apr. 11, 1930

Tube No.	Type of stopper	Date of first Appearance of growth	Approximate date of Presence of Considerable growth	Appearance of growth
H266	P*--2 hole	no growth		
H267	" "	"		
H268	" "	"		
Compound--Atropine sulphate.				
H263	" "	no growth		
H264	" "	"		
H265	" "	"		

EXPERIMENT 16.

As in experiment 15 the media used in this experiment have as a base 3% glycerol agar. However the added substances consist entirely of carbohydrates, which are present in a concentration of 1%.

Dextrose

The value of dextrose as a substitute for glycerol or as an aid to the growth of B. tuberculosis has been the subject of investigation by a number of workers. Proskauer and Beck (1894) found that in media containing both glycerin and glucose, the organisms grew profusely, while when the glycerin was absent no growth occurred. Dubois (1896) utilized a glucose-glycerin blood serum medium and obtained good growth. Matsuschita (1899) found that good growth resulted on a medium containing glucose glycerol, peptone and gelatin, while on a glycerol-free glucose peptone and agar the mammalian types of tubercle bacilli grew poorly. Avian types however, showed colonies of 1.5 mm. diameter after 6 days growth. Calmette, Massol and Breton (1909) concluded that media containing glucose without organic sources of nitrogen were not very satisfactory for cultivation. Kendall Day, and Walker (1914) were able to conclude that "dextrose plays no part apparently in the metabolism of the tubercle bacillus." Novy and Soule (1926) felt that while glucose might support the

growth of some strains, it was not the equal of glycerol. Henley (1929) found that dextrose was satisfactory for the cultivation of certain strains where it favored growth both in rate and total amount. This was confirmed by the work of Merrill (1931) who used four strains. Two of these (a bovine strain and one of the human strains, M) failed to show growth; one strain (H37) showed slow utilization of the glucose, while one saprophytic strain grew fairly well on a medium containing glucose but no glycerol.

EXPERIMENTAL

Inoculations were made in duplicate using 2 strains of human tubercle bacilli (H37 and Be) and one bovine strain (1698) onto slants of glycerol agar containing 1% glucose. The tubes were stopperd with cotton and paraffin-2 holes. As will be seen from Table 39 none of the tubes showed any growth after eight weeks.

Levulose

Proskauer and Beck (1894) found that media containing levulose but no glycerol gave evidence of some support of growth of B. tuberculosis. Wherry (1914) using a medium containing mineral salts, water and levulose, found that luxuriant growth resulted if KH_2PO_4 was present, but there was no growth if it was omitted. Merrill (1931) obtained results which indicated that only the saprophytic strain of four which he employed, would grow on levulose medium, confirming Kondo (1925).

EXPERIMENTAL (See Table 39)

Inoculations were made into tubes of levulose-glycerol agar, using the same strains as for dextrose, H37, B e , and 1698; these were in duplicate excpt H37 for which only one tube was inoculated, by error. Incubation at 37°C for eight weeks failed to demonstrate growth in any case. All tubes were paraffin-sealed with 2 holes.

III. Lactose

Proskauer and Beck (1894) were unable to grow the tubercle bacillus in media containing lactose, unless glycerol was also present. Merrill (1930)

EXPERIMENTAL

Duplicate inoculations, using the same strains (H37-1698, B e) were made in tubes of lactose glycerin agar, these were paraffin sealed and incubated at 37°C, but failed to show growth in any case after eight weeks, except in one tube of 1698 (D224) where a distinct growth was noticed by May 15, 1930. Apparently this culture could utilize lactose, but it was able to grow in spite of the presence of the glycerol and lactose.

IV. Galactose

Merrill (1931) stated that of his four cultures of B. tuberculosis only one the saprophytic strain, was able to grow on a glactose medium. The other three strains grew not at all.

EXPERIMENTAL

Of all the inoculations in duplicate using the three strains mentioned before, not one was positive after incubation of the glycerol-galactose agar at 37°C for eight weeks. Apparently galactose is not utilized by the organism and may actually have a sparing action, altho this experiment is not conclusive and should be repeated.

V. Raffinose

The failure of raffinose to serve as a substitute for glycerin in satisfying the carbohydrate metabolism requirements of the tubercle bacillus was indicated by Proskauer and Beck. Merrill likewise obtained negative results with all four of his strains, including the saprophytic strain.

EXPERIMENTAL (See Table 39)

Tubes of raffinose glycerinagar were inoculated with three strains of B. tuberculosis (2 tubes each with H37 and B e, three tubes with 1698). Although paraffin sealed and incubated for eight weeks at 37°C, growth failed to appear in any tube.

VI. Rhamnose

We are unable to find any record of work upon the utility of rhamnose as an adequate supply of carbon for the metabolism of the tubercle bacillus.

EXPERIMENTAL

Two tubes each of rhamnose-glycerin agar were inoculated with the three strains of tubercle bacillus used in this experiment. After being sealed with paraffin 2 holes, the tubes were incubated at 37°C. No growth was in evidence in any tube after 8 weeks of incubation. Since the glycerin was available to the organisms it seems that the carbohydrate (rhamnose) may exert a sparing influence on the glycerin-metabolism of the organism.

VII. Xylose

This pentose sugar has also been left untouched (so far as we can determine) in the search for suitable substitutes for glycerin in the metabolism of the tubercle bacillus. These pentose sugars were tried by us since it was resolved that if the tubercle bacillus could attack the three carbon molecule of glycerin, it might be able to utilize the five-carbon molecule of such sugars as xylose, rhammose, and arabinose. In higher forms of life certain analogous reactions take place where molecules containing even-numbered carbon groups maybe assimilated while odd-numbered carbon molcules are untouched or vice versa.

EXPERIMENTAL

Tubes of xylose glycerin broth were inoculated, two tubes each with H37 and B e and one tube with 1698. After sealing with 2-holed paraffin, incubation at 37°C was carried out. No growth occurred in any tube even after eight weeks incubation.

VIII. Mannose

Mannose was not assimilable by tubercle bacilli, as determined in 1894 by the experiments of Proskauer and Beck. No further work has been done with mannose, so far as we can discover. A number of investigators, however, have attempted to determine the nutritive value of mannitol (or mannite, as it is called), a closely related compound. Thus Proskauer and Beck (1894) found this compound, too, to be of no avail. Similar results were obtained by Kendall, Day and Walker (1914) Kondo (1925) obtained negative results, as did Merrill (1931) with all but his saprophytic strain.

EXPERIMENTAL

Two tubes each of mannose glycerin agar inoculated with three strains of B. tuberculosis (H37, Be, 1698). These were paraffin-sealed in the usual way and incubated at 37°C . After eight weeks no growth was evident in any tube.

IX. Maltose

Proskauer and Beck (1894) believed maltose to play no part in the metabolism of the tubercle bacillus since in the absence of glycerin media containing maltose were unable to sustain the growth of B. tuberculosis. Merrill(1931) concurred in this opinion since none of his four strains of tubercle bacilli would grow in his medium to which maltose had been added but from which glycerin was lacking.

EXPERIMENTAL

Two tubes each of three strains of B. tuberculosis (H37, Be, 1698) were prepared by inoculation into maltose-glycerin agar. No growth was observed in any tube after eight weeks, the tubes having first been paraffin-sealed with two holes and subsequently incubated at 37°C. These results bear out the work of the previous investigators.

X. Arabinose

As indicated previously very little work has been done with sugars, particularly the 5-carbon sugars. Kondo(1925) however, found that arabinose was of little value to the tubercle bacillus. He obtained the impression that a slight increase in size of inoculation took place on the first transplant onto arabinose-containing medium, but could not carry out further transplants from this. Merrill (1931) found that only his saprophytic strain would growin a medium containing arabinose but no glycerin.

EXPERIMENTAL

Two tubes each were inoculated with the three strains of B. tuberculosis used in this series of experiments, the medium being a 1% arabinose -3% glycerin agar. The tubes were sealed with paraffin in the customary way, then incubated at 37°C. After eight weeks incubation no growth was observed in any tube.

XI.Dextrin

Since the tubercle bacillus fares so well on starch and potato medium containing a high percentage of starch, it was considered whether or not the organism could grow on a medium containing lower homologues of the same group, such as dextrin. This subject has not been reported in the literature, except by Proskauer and Beck(1894) who noted slight growth in media containing it.

EXPERIMENTAL

Two tubes each were inoculated onto dextrin-glycerin agar, using the three strains of B. tub erculosis as previously. All tubes were paraffin-sealed and incubated at 37°C. One tube was contaminated by mold, while the others showed no evidences of growth even after eight weeks incubation.

XII.Salicin

Merrill (1931) found that salicin was of no value in the metabolism of the tubercle bacillus since none of his cultures were able to grow on a medium containing salicin but no glycerin. Likewise Model (1929) who worked with various capillary-active substances including salicin found that those which depressed the surface tension of the medium below 0.700 were able to inhibit the growth of B. tuberculosis. Salcin which weakly capillary active slowed the growth, but not completely inhibit it.

EXPERIMENTAL (See Table 39)

Three tubes of 1% salicin-3% glycerol agar were inoculated with H37 and after being paraffin-sealed, were incubated at 37°C. After eight weeks, two of the tubes failed to show any growth while the third gave evidence of slight, though distinct growth. We believe that the culture was able to utilize the glycerol of the medium, but could not attack the salicin; furthermore, the salicin exerted slight inhibitory effect on the organisms, since the growth was not as prolific as that on the control 3% glycerol agar.

XIII. Saponin

Dostal (1916) reported an interesting case in which his culture of B. tuberculosis after cultivation on saponin-containing media last its acid fastness, became (greasy butryous) ~~ordinary~~ in consistency like sarcinae, and grew on ordinary artificial media in a few days in the form of tender transparent layers. Schmurer (1922) in an endeavor to repeat this work was unable to do so and concluded that the results obtained by Dostal were due to a contamination of the subtilis-mesentericus group.

EXPERIMENTAL (See Table 39)

Of three tubes, containing 1% saponin - 3% glycerol agar inoculated with H37 and incubated at 37°C., after being sealed with paraffin- 2 holes, one was contaminated, while the other two showed slight growth, consisting of small isolated colonies. This was in contrast to the vigorous growth on the control tubes con-

taining three percent glycerin agar. It seems to indicate that the culture could utilize the glycerol of the medium for its metabolism but that the saponin exerted a slight degree of inhibition upon the growth.

XIV. Inulin

Merrill (1931) noted that none of his four strains of tubercle bacillus was able to grow on a medium which contained inulin but was glycerol-free, bearing out the observations of Proskauer and Beck.(1894)

EXPERIMENTAL

One tube of inulin glycerol agar was inoculated with H37 and after proper paraffin-sealing, was incubated at 37°C for 8 weeks. No growth was visible at the end of this period.

XV. Inosite

No work has thus far been reported on the availability of inosite to the tubercle bacillus for its metabolism. According to T.B.Johnson it is one of the chemical constituents of the organism.

EXPERIMENTAL(See Table 39)

One tube of inosite-glycerin agar was inoculated with H37 and incubated at 37°C for 8 weeks after being paraffin-sealed. No evidances of growth were observed during or at the end of this period.

XVI. Esculin

Although this interesting glucoside has found use in other fields of bacteriology such as the study of th colon-typhoid gray, we are unable to find any reference to its use in as a substitute for glycerol in serving as a source of carbon for the metabolism of the tubercle bacillus.

EXPERIMENTAL

One tube of esculin-glycerol agar was inoculated with H57 after suitable paraffin-sealing and incubation at 37°C, it was observed from time to time for signs of growth. No growth occurred after eight weeks incubation.

MEDIUM P

Sugar Glycerol Agar

1. A 3% glycerol agar was prepared in the usual way by adding to 1000 cc. H_2O (distilled)

NaCl	8.0 g.
Bacto agar	23.0 g.
Glycerol	30.0 g.

2. The hot mixture was filtered. It was then tubed, and to each tube (containing 9 cc. of glycerol agar) was added 1 cc. of a 10% solution of a sugar (see below) in water.
3. The tubes were shaken to mix the ingredients, and were then autoclaved for 25 minutes at 15# pressure, slanted and then allowed to cool.
4. The solutions of sugars were prepared by weighing out 1 g. of a sugar and adding this weight to 9 cc. of H_2O in a test tube, shaking and heating where necessary to dissolve the sugars. Then 1 cc. of the solution was pipetted into the tubes containing the hot agar mixture.
5. 10% solutions in water of the following substances were prepared.

a. dextrose
b. lactose
c. maltose
d. levulose
e. arabinose
f. xylose
g. galactose
h. mannose
i. dextrin
j. rhamnose
k. raffinose
l. Saponin
m. Esculin
n. Salicin
o. Inulin
p. Inosite

From three to six tubes of each sugar medium were prepared.

Experiment

The effect of carbohydrates on the growth of the tubercle bacillus.

(Medium T.)

Date of Inoculation--Mar.22, 1930

Dextrose		Levulose		Lactose		Galactose		Raffinose		Rhamnose	
H211	*	H219	-	H223	-	H213	-	H221	-	H209	-
H212	-	B219	c	H224	-	H214	-	H222	-	H210	-
B211	-	B220	-	B223	-	B213	-	B221	-	B209	-
B212	-	D219	-	B224	-	B214	-	B222	-	B210	-
D211	-	D220	-	D223	-	D213	-	D221	-	D209	-
D212	-			D224	!	D214	-	D222	-	D210	-
								D225	-		

Xylose		Mannose		Mātose		Arabinose		Dextrin		Salicin	
H207	-	H205	-	H203	-	H217	-	H215	c	H283	-
H208	-	H206	-	H204	-	H218	-	H216	-	H284	-
B207	-	B205	-	B203	-	B217	-	B215	-	H285	!
B208	-	B206	-	B204	-	B218	-	B216	-		
D207	-	D205	-	D203	-	D217	-	D215	-		
		D206	-	D204	-	D218	-	D216	-		

Saponin		Inulin		Inosite		Esculin	
H237	c	H202	-	H201	-	H295	-
H238	!						
H239	!						

c-----contamination
- No growth by May 15, 1930--indicates negative results.
! --Distinct growth by May 15, 1930

BLANK PAGE

Discussion

From the experiments which have just been reported certain general conclusions may be drawn and facts brought out.

(1) In our hands, media containing 6-carbon sugars have proved to be of no value in the metabolism of the tubercle bacillus. The sugars used show no growth-promoting properties and seem to display a distinct glycerol-sparing effect which inhibits growth. In some cases this is contrary to the results obtained by other workers. Varying results may be attributed in part at least to the different strains used.

(2) Five-carbon sugars are of equally little value in aiding growth of the organism; in these experiments, they too, have a glycerol-sparing effect, resulting in the inhibition of growth.

(3) The glucosides show interesting differences, Glucosides like esculin, seem to produce a complete inhibition of growth of the organism, even in the presence of glycerin. On the other hand salicin and saponin, while showing slight inhibitory powers when compared with control tubes of 3% glycerin nutrient agar, do permit some growth of the organism, as shown by our results.

(4) Dextrin seems to have no beneficial properties for the growth of B. tuberculosis.

(5) The value of media containing glycerol without other sugars is brought out clearly here, as well as in the other experiments which we have attempted to explain.

EXPERIMENT 17

In this experiment several well-known liquid media were used and an attempt was made to determine the presence of any relation between degree of oxygenation of the culture and the growth of the tubercle bacillus. For the latter purpose it was necessary to construct the apparatus shown in Figure 1 (see section on technique).

(s) Strain H_{37}

1. Using Glycerin nutrient broth

Flask numbers---H108---H101---H113

On Mar. 21 inoculations were made from 3% glycerol agar slant of H_{37} into two 250 cc. Erlenmeyer flasks containing 150 cc. of 3% glycerin nutrient broth each. Incubation was at 37°C. Flasks were oxygenated continuously (2 bubbles per second) from Mar. 28 to Apr. 4. They were then allowed to "rest" until Apr. 11, when oxygenation was begun again and carried on at the previous rate until May 1. On May 15 there was still no sign of growth and the results were interpreted as negative.

2. Calmette-Massol-Breton Medium.

Flask numbers---H102---H106---H107

Inoculation on Mar. 21 of 300 cc. Erlenmeyer flask containing 150 cc. of the above medium. Incubated at 37°C and oxygenated beginning Mar. 28. On Apr. 4, H102 and H107 had become contaminated by mold. H106 was oxygenated from Apr. 11, to May 1 continuously (at the rate of 2 bubbles per second). By May 15 there were yet no signs of growth and the results were interpreted as negative.

3. Medium of Tiffeneau and Marie.

Flasks numbers---H1030--H111

Inoculation on Mar. of two 500 cc. Erlenmeyer flasks containing 200 cc. of the above-named medium. Incubation at 37°C. H103 oxygenated continuously (2 bubbles per second) from Apr. 11-May 1. No growth by May 15. H111 oxygentaed from Mar. 21-Apr. 4 and from Apr. 11- May 1. No sign of growth by May 15.

4. Medium of Koch

Flasks numbers---H104---H112

Inoculation on Mar. 21 of two flasks containing 150 cc. of Koch's medium. Both were incubated at 37°C. from Mar. 21 to Apr. 11 without any apparent growth. They were oxygenated from Apr. 11- May 1 continuously. By May 15 there was still no sign of growth.

5. Medium of Proskauer and Beck.

Flasks numbers---H110

Inoculation on Mar. 21 of one 300 cc. Erlenmeyer flask containing 150 cc. of the medium of Proskauer and Beck. Incubated at 38°C. and oxygenated from Mar. 21-Apr. 4. Allowed to incubate until Apr. 11 when oxygenation was again commenced and continued until May 1. There was however, no sign of growth by May 25.

6. Beck's Medium

Flask numbers---H105---H109

Inoculation on Mar. 21 of two 300 cc. Erlenmeyer flasks containing 150 cc. each of Beck's medium. These were oxygenated from Apr. - May 1 while being incubated at 37°C. There was, however, no growth by May 15.

(b) Strain S_{1800}

1. Using Nutrient Glycerin Broth S_{105}

On Mar. 8 one flask (150 cc. Erlenmeyer) containing about 80 cc. of glycerin nutrient broth was oxygenated for 10 minutes with oxygen from the tank at the rate of 5 bubbles per second. Into this was inoculated a seeding of S_{1800}. The flask was then oxygenated once more by bubbling oxygen through for 10 min. at the same rate as previously. It was finally shaken vigorously and placed in the 37°C., incubator. On Mar. 14 it was oxygenated again for one hour at the previous rate and was then returned to the incubator. The experiment was terminated by the contamination of a mold growth on Mar. 21.

2. Medium of Calmette-Massol-Breton

Flask number---S101

On Mar.8 one Erlenmeyer flask (300 cc. capacity) containing about 150 cc. of medium of Calmette-Massol-Breton was oxygenated for about 15 minutes using a tank of oxygen and bubbling it through at the rate of about 4 bubbles per second. Into this was inoculated a seeding of Strain S_{1800} The flask was then oxygenated once more by bubbling at 5 bubbles per second for ten minutes and after being thoroughly shaken was placed in the incubator. On Mar. 13 it was removed and aerated 1 hour at 5 bubbles per second , then reincubated. On Mar. 14 it was found contaminated by mold.

3. Koch's Medium

Flask number---S104

On Mar. 8 a 500 cc. Erlenmeyer flask containing about 200 cc. of Kock's medium was oxygenated for five minutes at the rate of 5 bubbles per second. Into this was inoculated strain S_{1800} The flask after being reoxygenated for ten min. at the previous rate and thoroughly shaken was placed in the Incubator. On Mar. 13 and Mar. 14 it was aerated for fifteen min. and one hour respectively at the rate mentioned above and returned to the incubator. On Mar. 21 it was oxygenated at one bubble per second continuously until Apr. 4 while in the incubator. After incubation without

oxygenation until Apr. 11 it was re-oxygenated from Apr. 11 to May 1. There was no sign of growth by May 25.

4. Beck's Medium

On Mar. 8 a 250 cc. Erlenmeyer flask containing about 150 cc. of Beck's medium was oxygenated for about 10 min. at a rate of 4 bubbles per second. Into this was inoculated strain S_{1800}. The flask was then re-oxygenated for ten minutes at 5 bubbles per second. It was then shaken and placed in the incubator. On Mar. 13 it was oxygenated 15 minutes at 5 bubbles per second and incubation continued. On Mar. 14 it oxygenated one hour at the above rate. Mar. 21 it was oxygenated continuously at one bubble per second until Apr. 4. From Apr. 4 to May 11 it was oxygenated continuously at a rate of one bubble per second. There was no growth by May 25.

MEDIUM Q

Koch's Medium

Asparagin	5.0 g.
KH_2PO_4	5.0 g.
Glycerol	20.0 g.
$MgSO_4$	1.5 g.
Na citrate	2.0 g.
NaOH	2.5 g.
Distilled H_2O	1000. 0 cc.

These substances are added to the distilled water and dissolved. The solution is filtered, autoclaved 20 minutes at 15# pressure and is ready for use.

MEDIUM R

Beck's Medium (Calmette--p.34)

1. Beck uses 100 cc. of serum (from horse, ox, or swine) which he adds to 900 cc. of water and heats to boiling for one hour (in an unbolted autoclave).
2. After filtration he adds the following to the clear fluid:-

Cit rate of magnesia	2.5g.
Asparagin	2.0g.
Glycerol	20.0g.

3. He then autoclaves the mixture once more for 15 to 20 minutes at 112°C. and filters. The broth is portioned out in flat-bottomed flasks containing fluid to a depth of 2 cm. The flasks are then plugged with cotton and sterilized for half an hour in the autoclave at 120°C.
4. After cooling, the flasks are ready to be inoculated.

Our Modification (Medium R)

1. Bacto Loeffler's Blood Serum (Digestive ferments Co.) (containing 3 parts blood serum and 1 part dextrose broth) 8.0 g.

$MgSO_4$	1.0 g.
Na citrate	2.0 g.
Glycerol	20.0 g.
Distilled H_2O	1000.0 cc.
Asparagin	2.0 g.

2. The dehydrated serum was added to the water, the mixture boiled ten minutes and filtered. To the filtrate was added the $MgSO_4$, Na citrate, glycerol, and asparagin.
3. After shaking to dissolve the salts, the mixture was autoclaved 20 minutes at 15# pressure.
4. The medium was portioned out into flat-bottomed Erlenmeyer flasks to a depth of about 3/8". The flasks were then autoclaved again at 15# for 25 minutes. The cooled and were ready for use.

MEDIUM S

Medium of Proskauer & Beck

K_2CO_3	3.5 g.
$(NH_4)\ H_2PO_4$	2.5 g.
$MgSO_4$	2.5 g.
Glycerol	20.0 g.
Distilled H_2O	1000.0 cc.

The salts are dissolved in water; the glycerol is added and the whole autoclaved. It is allowed to cool. (Autoclaving for 20 minutes at 15# pressure).

MEDIUM T.

Medium of Tiffeneau & Marie.

KH_2PO_4	5.0 g.
$MgSO_4$	2.5 g.
Mannite	6.0 g.
$(NH_4)_2SO_4$	2.0 g.
Glycerol	15.0 g.
Distilled H_2O	1000.0 cc.

The salts are dissolved in the water; the sugar is added and dissolved; the glycerol is added finally with stirring, and the completed medium is autoclaved at 15# pressure for 20 minutes. It is then allowed to cool.

Medium U.

Medium of Massol, Breton, and Calmette.

Na_2CO_3	1.0 g.
$FeSO_4$	0.040 g.
$MgSO_4$	0.050 g.
K_3PO_4	1.0 g.
NaCl	8.5 g.
Glucose	10.0 g.
Glycerol	40.0 g.
Witte's Peptone (Difco used)	10.0 g.
Distilled H_2O	1000.0 cc.

The salts are disolved in water, then the glucose and peptone are added. Finally, the glycerol is dissolved. The medium is filtered and autoclaved for 20 minutes at 15# pressure. It is then allowed to cool.

SUMMARY

In an effort to correlate, in so far as the date would permit, the results of the experiments presented here, the following table (Table 40) was constructed. The numerals signify the number of weeks required for any given strain to present growth on any particular given medium. Zero denotes failure to produce growth.

It is evident from the tabulated record that the length of time required for growth to appear in any given strain varied (with the exception of strain "54") considerably with the type of medium used. H37 appears to have given growth in the majority of instances in about four or five weeks. Strain "54" gave fairly good growth in nearly all the media on which it was cultivated in one week. Strain G624 gave uniformly negative results with the exception of 3% glycerol agar. Strain S_{1800} an exceedingly virulent organism grew in about two to seven weeks depending on the medium used. Strain 1698 grew on most media in five weeks but on egg media growth odcurred in two instances within a week of the time of inoculation. About six weeks or more were required for T_{271} to exhibit growth.

TABLE 40

Medium	H37	54	Be	G624	S1800	1698	T271
3% Glycerol Agar	6	1	6	9	2	5	6
Percents of Glycerol	0	3	0	0	0	0	0
no glycerol							
1% "		1			7		0
2% "		1					0
3% "		1					
4% "		1					
5% "							
6% "		1					
7% "		1					
8% "		1					
9% "		1					
10% "		1					
Glycerol Nutrient Broth.							3
Potato Extract Glycerol Agar	10	1	10	0	6	6	10
Glycerinated Potato	3	1	3	0	8	5	5
Gentian-Violet-Potato Juice Agar	4	1	2	0	3	5	0
Potato-egg-glycerol medium	2				0	0	
Petragnani's medium	1					1	
Corper's medium	1		0		0		
Petroff's medium	2				0	0	0
Dorset's medium							
(no glycerol 0	0				0	0	0
(3% glycerol 1	1				1	1	

TABLE 40 (cont)

6

Medium	H37	54	Be	G624	S1800	1698	T271
Carbohydrates							
Dextrose	0		0			0	
Levulose	0		0			0	
Lactose	0		0			7	
Galactose	0		0			0	
Raffinose	0		0			0	
Rhamnose	0		0			0	
Xylose	0		0			0	
Mannose	0		0			0	
Maltose	0		0			0	
Arabinose	0		0			0	
Dextrin	0		0			0	
Salicin	7		0			0	
Saponin	7		0			0	
Inulin	0						
Inosite	0						
Esculin	0						
Protein substances							
Egg albumin	3						
Casein	4						
Gelatin	3						
Amino acids							
Glycine	4						
Leucine	4						
Asparagin	4						
Tissue and tissue extracts	0	0	6	0	0	0	0
Hesse's medium	7	1	6	0	2	4	5
Creatin	2						
Creatinin	2						

TABLE 40 (cont.)

Medium	H37	54	Be	G624	S1800	1698	T271
Cholesterol	3						
Pancreatin	3						
Pepsin	3						
Na glycerophosphate	4						
Ca saccharate	5						
Na taurocholate	5						
Oxgall	0						
Alkaloids							
Brucine	0						
Atropine sulphate	0						
Benzene derivatives							
Vanillin	0						
Orcinol	0						
Na benzene sulphonate	6						
Na sulfindigotate	4						
Medium of Besredka and Jupille	0						
Increased oxygen							
Glycerol nutrient broth	0						
Calmette-Massol-Breton	0						
Tiffeneau and Marie	0						
Koch's medium	0						
Proskauer and Beck	0						
Beck's medium	0						

CONCLUSIONS

1. A comprehensive investigation of the chemical, physical and biological forces which may influence the cultivation of the tubercle bacilli on artificial media is presented.

2. Koch's pessimistic prophecy " that it is not to be hoped that the cultivation of the tubercle bacillus is to play any very important part in the study of the disease" has not been fulfilled in the light of investigations of the past fifty years.

3. No medium has as yet developed which can be described as definitely superior to all others for the cultivation of the tubercle bacillus. Such a condition is perhaps hardly to be hoped for since the numerous factors involved render a complete study an impossibility.

4. The most promising media for the rapid and certain cultivation of the tubercle bacillus are those in which glycerol, eggs, or potato, predominate.

5. For certain definite purposes some media seem to be more suitable than others. (see below)

6. Glycerol agar or glycerol broth may be used for the initial cultivation of tubercle bacilli from various sources. The best concentrations of glycerol are probably those from 3 to 6%. Higher concentrations may inhibit growth.

7. Potato media are useful in making serial cultures and for storing the organism.

8. Egg media are very good for obtaining initial growth after suitable preliminary treatment to destroy contaminants.

9. Potato and egg combinations offer interesting possibilities and should be investigated further.

10. Mineral salts, if not in too high a concentration and in the presence of glycerin, may aid growth. Phosphorus, sulphur, potassium and magnesiun are indispensable to the growth of B. tuberculosis.

11. Carbohydrates as a rule, do not exert a beneficial action on the growth of the tubercle bacillus.

12. Tissue media have not, with some exceptions, yielded any valuable results.

13. Dyes in media are useful for inhibiting growth of extraneous organisms but care should be taken that in preparing the medium lethal concentrations for the tubercle bacillus are not produced in any portion, particularly the surface of the medium.

14. The optimal temperature for cultivating the tubercle bacillus of mammals is 38°C.

15. An important phase of the cultivation of the tubercle bacillus is the prevention of dehydration of the medium.

16. A sufficient supply of oxygen must, as a rule, be supplied the organism. However, continuous oxygenation of the culture is probably useless or even worse.

17. A concentration of carbon dioxide of 5.5% definitely inhibits growth of the tubercle bacillus.

18. The best reaction of a medium for good growth depends on the type of organism to be inoculated; the human type appears to thrive best in an acid medium, whereas the bovine type probably prefers an alkeline environment.

19. Substances capable of lowering the suface tension, if not toxic in themselves, usually aid the growth of B. tuberculosis.

20. Culture tubes when exposed to sunlight passing through the glass are sterilized in 1 to 1½ hours. Precautions should therefore be taken to prevent prolonged exposure.

21. The most rapid growth usually occurs where there is a continuous succession of transplants on the same medium.

22. The acid-fast characteristics may be altered by growth on media containing cod-liver oil or those lacking glycerol.

23. The virulence of the tubercle bacillus may be maintained by growth on glycerol potato or egg media, but transplants should be made every three or four weeks on fresh media. Growth on potato-bile decreases or perhaps destroys the virulence of tubercle bacilli. The solid culture media, particularly glycerol potato, sustain the virulence of the bacillus much better than do the fluid media.

24. The chemical composition of B. tuberculosis can be ascertained to a large extent by cultivation on media of definite chemical composition.

25. Only from cultures on fluid media can tuberculin be prepared and the soluble excretory poisons of the bacillus studied.

26. For isolation purposes, cultivation on artificial media may in time entirely displace the guinea pig inoculation method. In isolation work, the more inoculations that are made the better are the chances of securing growth and avoiding false negatives. Contamination, however, is still a factor to be dealt with in spite of the fact that considerable success has already been attained in combatting the difficulty.

BIBLIOGRAPHY

Alasandrini, A.: Culture of tubercle bacillus using glycerin potato medium. Policlinico Sez. prat. 866, 1926.

Alilaire, et Fernbach: Quelques obsevations sur la culture du bacille tuberculeux en milieu non glycerine. C. R. Acad. des Sci. 171: 375, 1920.

Allen, S. M.: On Corper's new culture method for the tubercle bacillus. Jour. Mental Sci. 75: 690, 1929.

Anzilotti, J.: Uber ein besonderes kulturs enfahren fur den tubercelbacillus auf Kartoffeln. Centr. f. Bakt. 40: 765, 1906.

Arloing, F. and Malaitre: Aspects des coupes des cultures de bacilles tuberculeux sur pomme de terre. Presse Med. 53: 893, 1925.

Arloing, F. and Dufourt, A.: Polymorphisme du bacille tuberculeux: formes myceliennes ramifiees. Presse Med. 53: 896, 1925.

Arloing, F. and Dufourt, A.: Essai de cultur du bacille tuberculeux en eau ordinaire et en eau distillee. Compt. Rend. Soc. Biol. 98: 37, 1926.

Arloing, F. and Dufourt, A.: Morphologic variations of Tb. and the factors which occasion. (Effect of media on appearance). Presse Med. 773, 1927.

Arloing, F. and Dufourt, A.: Cultures du bacille tuberculeux en eau ordinaire et eau distillee. Presse Med. 10, 1928.

Armand-Delille, P.: Mayer, A., Schaeffer, G., and Terroine, E.: Peptone components necessary for growth of the tubercle bacillus. Comp. Rend. Acad. des Sci. 154: 537, 1912.

Armand-Delille, P., Mayer, A., Schaeffer, G., and Terroine, E.: Growth of tubercle bacillus on a medium of definite chemical composition. Comp. Rend. Soc. de Biol. 74: 274, 1913.

Bachman, R. and Poire, A.: La seroraction avec l'antigene de Besredka pour le diagnostic de la tuberculose. Compt. rend. Soc. de Biol., 88: 386, 1923.

Bang, D. L.: Vergleichende Untersuchungen uber den Wert der Eier-Nahrboden. Beitr. klin Tuberk., 75: 367, 1930.

Baudran, G.: Milieux artificiels pour la culture du bacille de Koch. Presse Med., 891-892, 1909.

Baudran, G.: Milieux artificiels attenuant ou exaltant la virulence du bacille de Koch. Compt. rend. Acad. des Sci., 149, 1909.

Baudran, G.: Bacilles de Koch. Milieux aux glycerophates. Doses maxima de fer et de manganese. C. r. Acad. des Sci., 150: 19: 1200-02, 1910.

Becquerel, P.: Influence des sels d'uranium et de thorium sur le developpement du bacille de la tuberculose. Compt. r. Acad. des Sci., 156: 164, 1913.

Besancon, P. et Boudin: Influence de la composition des milieux sur le developpement der cultures du bacille de Koch. Bull. de la Soc. d'etudes scient. sur la Tub. 3: 1-8, 1913.

Bezancon, F.: L'analyse chimique du bacilles tuberculeux et les milieux de culture. Rev. d. l. Tuber., 4: 397, 1923.

Bezancon, F. and Griffon, V.: Culture du bacilli tuberculeux sur la jaune d'oeuf gelose. Compt. rend. Soc. de Biol., 55: 603, 1903.

Besredka, A.: Culture des bacilles tuberculeux dans du jaune d'oeuf. Ann. de l'inst. Pasteur. 35: 291, 1921.

Besredka, A.: Culture des bacilles tuberculeux dans du jaune d'oeuf. Ann. de l'Inst. Pasteur. 35: 291, 1921.

Besredka, A. and Jupille, F.: La gelose a l'oeuf. Ann. de l'inst. Pasteur. 28: 576, 1914.

Besredka, A. and Jupille, F.: Study of the tubercle bacillus Compt. rend. Acad. des Sci. 156: 1633, 1913.

Besredka, A. and Jupille, F.: Ein neuer Nahrboden fur Tuberkelbazillen. Zeit. f. Tuberkulose. 21: 53, 1913.

Besredka, A. and Jupille, F.: Egg bouillon. Ann. de l'Inst. Pasteur. 27: 1009, 1913.

Bindo de Vecchi: I tessúti, animali come substrati nutrivi per il bacillo tubercolare. Rif. med. 29: 1906.

Blechmann, M.: Etude comparative de la valeur des milieux de Petroff et de Petragnani pour l'isolement et la culture du bacille de Koch a partir des crachats tuberculeux. Compt. rend. Soc. de Biol. 96: 1033, 1927.

Boecher, E.: Uber das Wachstum von Tuberkelbacillen in eidotterhaltiger flussigen Nahrboden. Ztschr. f. Hyg. 95: 344, 1922.

Boecher, E.: Uber die subnerse Vermehrung der Tuberkel-bacillen in flussigen Nahrboden. Ztschr. f. Hyg., 99: 121, 1923.

Boez, L.: Peptone culture medium for Bacillus tuberculosis. Ann, Inst. Pasteur 40: 746-754, 1926.

Boissevain, C. H.: The action of unsaturated fatty acids on tubercle bacilli. Am. Rev. Tuberc. 1: 13: 84-89, 1926.

Boissevain, C. H.: A method for obtaining single colonies of tubercle bacilli. Am. Rev. Tuberc. 1: 13: 90-91, 1926.

Borrel, A., de Coulon A., Boez, L., et Quimaud, J.: Milieu synthetique pour la culture du bacille tuberculeux. Compt. rend. Soc. de Biol. 86: 388, 1922.

Borrel, A., Boez, L., and de Couon, A.: Racteurs accessoires de bacille tuberculeux. Compt. rend. Soc. de Biol. 89: 191, 1923.

Bossan, E. and Baudy, M.: Nouveau procede d'isolement du bacille tuberculeux dans les crachats. Compt. rend. Soc. de Biol. 87: 954, 1922.

Bouquet, A.: Differentiation of human from bovine tubercle bacilli by use of arbutinine. Rev. de la Tuberc. Ser. 3: 2: 485-494, 1921.

Braun, H. and Kondo, S.: Der Verwendungsstoffwechsel des Tuberkelbacillus. Klin. Wocknschr. 3: 10, 1924.

Brechmann, H. J.: Demonstration of tubercle bacilli, especially by the culture method. Zentralbl. Bakt. I. Abt. Orig. 111: 26-31, 1929.

Brooks, M. M.: The effect of hydrogenion concentration on the production of carbon dioxide by the tubercle bacillus. Am. Rev. Tuberc. 5-6: 369-376, 1922.

Brown, L. and Smith, D.: Cultivation of tubercle bacilli directly from sputum by the use of anti-formin. J. Med. Res., 22: 517, 1910.

Browning, C.H. and Gulbrausen, R.: The acid-fast property of the tubercle bacillus. J. Path. and Bact. 27: 326, 1924.

Buc, E.: Sur la relativite du caractere aerobie du bacille Tuberculeux. Compt. rend. Soc. de Biol. 88: 1135, 1923.

Buc, E.: Sur la croissance du bacille tuberculeux dans les milieux liquides. Rev. d. l. Tuberc. 5: 520, 1924.

Buc, E.: Procede de culture du bacille de Koch dans les epanchements pleuraux. Compt. rend. Soc. de Biol. 98: 1495, 1928.

Burn, C.G.: Dorset's egg medium for the cultivation of tubercle bacilli, and its modifications. Am. Rev. Tuberc. 6-(10): 950-59, 1922.

Calcaterra, L.: Lisi bacillari da lecitin. Ann. dell' Istit. Maragl. 6: 12, 1912.

Calmett, A. and Guerin, C.: Bile potato medium for cultivation of tubercle bacillus. Compt. rend. Acad. des Sci. 147: 1456, 1908.

Calmette, A. and Limousin, H.: Milieu de Petroff et diagnositc bacteriologique rapide de la tuberculose des voies urinaires. Compt. Rend. Soc. d. Biol., 86: 935, 1922.

Calmette, A., Boquet, A., and Negre, L.: Quelques experiences relatives a la action de la sanocrysine sur le bacille tuberculeux et sur la tuberculose experimentals des rongeurs. Rev. d. l. Tuberc. vii, 1926.

Calmette, A., Boquet, A., and Negre, L.: Contribution a l'etude du bacille tuberculeux bilie. Ann. de l'Inst. Pasteur 35: 561, 1921.

Calmette, A., Massol, L., and Breton, M.: Liquid medium for abundant growth of tubercle bacilli. Compt. rend. Soc. de Biol. 67: 580, 1909.

Campbell, H.B., and Kieffer,: The action of codliver oil on the tubercle bacillus. Am. Rev. Tuberc. 6(10): 938-949, 1922.

Campbell, L. K.: The alanine and histidine metabolism of the tubercle bacillus. Am. Rev. Tuberc. 11-(5): 458-470, 1925.

Campbell, L. K.: Studies on the amino-acid content of the tubercle bacillus. Am. Rev. Tuberc. 11(5): 452-457, 1925.

Campbell, L. K.: On the production of acid-fastness in non-acid-fast bacilli. Am. Rev. Tuberc. 11(5): 450-451, 1925.

Cancik,J. A., and Mazepora, N.: Direct cultivation of the Mycobacterium tuberculosis. Am. J. of Pub. Health. 679, 1925.

Cantani, J. A.: Zur Verwendung des Sperma als Nahrbodung-usatze. Centr. f. Bakt. 22: 601, 1897.

Capaldi, A.: Zur Verwendung des Eidottera als Nahrboden-zusats. Centr. f. Bakt. 20: 800, 1896.

Churchman, J.W.: The effect of slight increase of temp-erature on the bacteriostatic power of gentian violet. Bull. John Hopkins Hosp. 33: 227, 1922.

Charpentier,: Bacilles tuberculeux et arsenic. Ann. de l'Inst. Pasteur. 29: 443, 1915.

Cobbett, L., Griffith, A.S., and Griffith, F.: Royal commission on tuberculosis (human and animal) Second interim report of the Royal Commission appointed to inquire into the relations of human and animal tuberculosis. Appendix. 3: part 2.

Cooper, F.B.: Influence of surface tension on the growth of the tubercle bacillus. Am. Rev. Tuberc. 21(3): 354, 1930

Corper, H.J.: The virulence of t he tubercle bacilli isolated from the sputum. J. Infedt. Dis. 23: 493, 1918.

Corper, H.J.: The cultivation of recently isolated and laboratory strains of human tubercle bacilli on artificial media. Am. Rev. Tuberc., 3(8): 461-472, 1919.

Corper, H.J.: Are all the tubercle bacilli found in the sputum virulent. J. Am. M. Ass. 70(918): 1281, 1918.

Corper, H.J., Fiala, L., and Kallan, L.: The routine cultivation of tubercle bacilli from the sputum by Petroff's method. J. Infect. Dis., 268, 1918.

Corper, H.J., and Gauss, H.: The preservation of cultures of human and bovine tubercle bacilli. Am. Rev. Tuberc., 6(11): 1040-1045, 1923.

Corper, H.J., and Uyei, N.: The isolation of tubercle bacilli from contaminated tubercuous materials. Am. Rev. Tuberc., 16: 299, 1927.

Corper, H.J., and Uyei, N.: Cultivation of the tubercle bacillus. An improved method for isolation from tuberculous materials. J.L. and Clin. Med. 13(5): 469, 1928.

Corper, H.J., and Uyei, N.: Further observations with a new method for cultivating tubercle bacilli: a comparison with guinea-pig inoculation and Petroff's method. J. Lab. and Clin. Med. 14: 393, 1929.

Corper, H. J., and Uyei, N.: A simple glycerol water crystal violet potato cylinder medium for diagnostic cultures of Tubercle bacilli. Arch. Path. and ab. Med. 7: 835, 1929.

Corper, H.J., and Uyei, N.: The ce rtified diagnosis of tuberculosis: Practical evaluation of a new method for cultivating tubercle bacilli for diagnostic purposes. J.A.M.A. 91: 371, 1928.

Corper, H.J., and Uyei, N.; Oxalic acid as a reagent for isolating tubercle bacilli, and a study of the growth of acid-fast non-pathogens on different media with their reaction to chemical reagents. J. Labo and Clin. Med. 15(4): 348, 1930

Corper, H.J., and Sweany, H. C.: The enzymes of the tubercle bacillus. J. Bact. 3: 129, 1918.

Corper, H.J., and Lurie, M.B.: The variability of localization of tuberculosis in the organs of different animals. The importance of the distribution of tubercle bacilli as concerns differences of susceptibility of the organs. Am. Rev. Tuberc. 14(6) : 680-705, 1926.

Corper, H.J., Simmons, J.S., and Freed, H.: The presence of virulent tubercle bacilli in human bile. Am. Rev. Tuberc. 8(3) : 260-265, 1923.

Corper, H.J., Gauss, J. and Rensch, O.B.: Studies on the influences of carbon dioxide on resistance to tuberculosis. The effect of carbon dioxide on the tubercle bacillus. Am. Rev. Tuberc. 5(7): 562, 1921.

Corper, H.J., Starry, A.C., and Lurie, M.B.: The effect of carbon on the tubercle bacillus in vitro and on experimental tuberculosis in the rabbit. Am. Rev. tuberc. 8(6) : 530-53, 1924.

Courmont, P., Morel, A. et Bay, L.: Sur le pouvoir infertilisant de quelques vegetables et de leurs constituants vis-a-vis des cultures homogenes de bacille tuberculeux humain. C.R.Soc. Biol. 98: 318, 1926.

Courmont, P., Morel, A. et Day, I.: Sur les modifications de la reaction et de l'azote ammoniacal dans les cultures homogenes de bacille tuberculeux humain A en milieu define a base d'ammoniaque. C.R. Soc. Biol. 95: 41, 1926.

Courmont,P., Morel, A. et Bay, I.: Sur les cultures homogenes de bacilles tuberculeux humain A en milieu peptone a reaction acide des le debut. C.R. Soc. Biol. 95: 831, 1926.

Cruickshank,J.: On the direct cultivation of tubercle bacilli from tuberculous tissues. Centr. f. Bakt. Abt. I Ref. 58: 43, 1913.

Cunningham, J.S. and Cummings, P.L.: Application of the Corper-Uyei culture method in the routine examination of sputum for tubercle bacilli. J.Lab. and Clin. Med. 15(6): 572, 1930

Cunningham,J.S.:Do the tubercle bacilli contain vitamins A and B. Am. Rev. Tuberc. 9: 487, 1924.

Dalimier, R., and Lancereau, E.: Le milieu de culture d'acides amines complets pour les microorganismes. Presse Med. 21: 419, 1913.

Despeignes, V.: Sur le diagnostic rapide de la tuberculose des voies urinaires sansinoculation au cobaye. Nouveau medium de culture plus rapide. Compt. rend. Soc. d. Biol. 87: 119, 1922.

Despeignes, V.: Application au diagnostic de la meningite tuberculose des milieux de culture electeif's pour le bacille de Koch. Compt. Rend. Soc. d. Biol. 87: 121, 1922.

Despeignes, V.: Diagnostic rapide de la tuberculose des voies urinaires sans inoculation au cobaye. Compt. Rend. Soc. de Biol. 86: 931, 1922.

DeWitt, L.M., annd Wells, H.G.: An attempt to establish tissue-specific strains of tubercle bacilli. Am. Rev. Tuberc. 13(1): 92-93, 1926.

Deycke, G. and Voigtlander,: Studien Uber kulturelle Nahrboden. Centr. f. Bakt. 29: 617, 1901.

Dienes, L.and Schoenheit, E.W.: A study of the nature of the specific substances in broth-culture filtrates of tubercle bacilli. Am. Rev. Tuberc. 11(2): 146-50, 1925.

Distaso, A.: Sur des milieux de culture liquides et solides prepares avec le serum digere et dilue. Compt. rend. Soc. d. Biol. 79: 599, 1916.

Dorset, M: The use of eggs as a medium for the cultivation of bacillus tuberculosis. Am. Med. 3: 555, 1902.

Dorset, M. and DeSchweinitz,E.A.: The growth of the tuberculosis bacillus on acid media. Dept. of Agric. Bur. Animal Industry. Bull., (13)

Dostal,H.: Die Glykosidform des Tuberkelbazillus. Frankf. Ztschr. f. Path. 19: 198, 1916.

Dubois,L.A.: Sur un nouveau mode de bacilli Koch. Comp. rend. Soc. de Biol. 48: 204, 1896.

Eastwood,A.: Royal Commission on tuberculosis. (Human and animal) Second interim report of the Royal Commission appointed to inquire into the relations of human and animal tuberculosis. Part II. Appendix. 4:

Editorial: Culture methods for the isolation of the tubercle bacillus. J. Lab. and Clin. M. 3(3): 207, 1917.

Endo, G.: Nahrboden fur Tuberkelbacillen. (Mitteil. der med. Gesellschaft zu Tokio. 16(77): 178, 1902.

Engel,W.:Zum kulturellen Nachweis von Tuberkelbacillen. Deutsche med. Wochnschr. 53: 1001, 1922.

Ficker, M.: Wachstum der Tuberkelbacillen auf sauren Gehirn-nahrboden. Centr. f. Bakt. 27: 504, 1900.

Ficker,M.: Wachstum der Tuberkelbacillen auf sauren Gehirn-nahrboden. Centr. f. Bakt. 27: 591, 1900.

Fraenkel, C.: Synthetic medium for cultivation of the tubercle bacillus. Hygienische Rundschau 17: 769, 1894.

Friedland,M.:Zur Frage des Einflusses der Strahlen-energie auf das Wachstum der Tuberkelbacillen in vitro. Centr. f. Bakt. 95: 404, 1924.

Frongia, E.:Influence of Malachite green on the growth of tubercle bacilli in pure culture. Giorn. d. Batteriol. e Immuno. 10: 705, 1927.

Frothingham,L.: The glycerine bouillon curve of tubercle bacilli as recorded by the Bovie Potentiometer. J. Med. Research. 37: 269, 1917.

Frothingham,L.:Brom Cresol Purple and litmus as indicators for the classification of tubercle bacilli. J. Med. Research, 39(2): 153, 1918.

Frouin, A.: Variation des matieres grease du bacille tuberculeux cultive sur milieux definis en presence de terres du groupe cerique. Comp. rend. des Sci., 170: 1471, 1920.

Frouin,A.: Culture du bacille tuberculeux sur la glucosamine et la sarcosine associees. Comp. rend. Soc. de Biol. 68: 915, 1910.

Frouin, A.: Developpement du B. Tuberculeux, type humain, sur milieu chimiquement defini en presence de sucres et de terres du groupe cerique. Comp. rend. Soc. de Biol. 83: 754, 1920.

Frouin, A.: Action of vanadium and rare earth salts on the development of the tubercle bacillus. Comp. rend. Soc. de Biol. 72: 1034, 1912.

Frouin, A.: Action des sels de terres rares sur le developpement du bacille tuberculeux et de l'Aspergillus niger. Comp. rend. Soc. de Biol., 73: 640, 1912.

Frouin, A.: Culture du bacille tuberculeux sur des mileux renferment quatre, six ou huit grammes de soude par litre. Comp. rend. Soc. de Biol. 74: 1184, 1913.

Frouin, A.: Influence of uranium and thorium salts on development of tubercle bacilli. Comp. rend. Soc. de Biol. 74: 282, 1913.

Frouin, A.; et Agulhou, H.: Action favorisante des sels de terres rares sur le developpement du bacille tuberculeux. Comp. rend. soc. de Biol. 78: 129, 1915.

Frouin, A., and Guillaumie,M.: Nutrition minerale du bacille tuberculeux. Action favorisante ou empechante des sels de terres rares. Comp. rend. Soc. de Biol. 89: 382, 1923.

Frouin, A. and Guillaumie, M.: Absence d'acides gras libre dans le bacilli tuberculeux. Comp. rend. Soc. de Biol. 89: 319, 1923.

Frouin, A. et Guillaumie, M.: Influence de la concentration minerale du milieu et de l'age des cultures sur la teneur en cendres du bacille tuberculeux. Comp. rend. Soc. de Biol. 94: 1340, 1926.

Frouin, A. and Guillaumie, M.: Influence of the concentration of glycerol in culture media on the weight increment of tubercle bacilli. Comp. rend. Soc. de Biol. 90: 731-32, 1924.

Frouin, A. and Guillaumie, M.: Influence des sels de fer sur le rendement en poids du bacille tuberculeux. Action de ces sels sur l'utilisation de la glycerine. Comp. rend. Soc. de Biol. 90: 831, 1924.

Frouin, A. and Guillaumie, M.: Influence de la concentration du phosphore et du potassium sur le developpement du bacille tuberculeux. Comp. rend. Soc. de Biol. 94: 1115, 1926.

Frouin, A. et Guillaumie, M.: Culture du bacille tuberculeux sur milieux synthetiques. Essai de generalisation de la notion d'equilibre entre les elements constit tifs des milieux de culture. Ann. Inst. Pasteur 42: 667-694, 1928.

Frugoni, C.: Uber die Kultivierbarkeit von Koch's Bacillus auf tierischen Gewebe. Centr. f. Bakt. 53: 553, 1910.

Gamble, C.J., and Herrick, M.C.: The utilization of dextrose by the tubercle bacillus. Am. Rev. Tuberc., 6(1): 44-50, 1922.

Gessard, G.: and Vaudremer,A.: Divers modes de culture du bacille tuberculeux. Compt. rend. Soc. d. Biol. 87: 1012, 1922.

Gessard, G.: et Vaudremer, A.: Recherches sur la culture du bacille tuberculeux. C. R. Soc. Biol. 90: 732, 1924.

Giese, C.: Experimentalle Untersuchungen uber die Einwirkung von organen, Organextacted, Exsudaten und sekreten auf Tuberkelbacillen im Reagenzglasse und entsprechende Heilund immunisierungsversuche gegen die Tuberkulose der Haustiere. Zeitschr. f. Immunitatsforsch. j. exper. Therap. 31: 533, 1921.

Gioelli, P.: Nuoui mezzi di rapido di sviipuppo del bacillo della tubercolosi di uno liquido non glicerihato. Polici. 14: 118, 1907.

Gioelli, P.: Ulteriore richerche sullo sviluppo virulenza del bacillo della tubercolosi coltivato in nuoni mezzi di cultura. Boll. d.r. accod. med. di Genova. 23: 2, 1908.

Gloyne, S.R.: A note on the viability on acid fast bacilli. Tuberc. ii, 12, 190.

Goeckel, J.J.: A convenient method for concentrating and isolating tubercle bacilli. Med. Res. 96: 804, 1919.

Goodman, F.L., and Moore, M.: Cultivation of tubercle bacilli. Jour. Infect. Dis. 31: 58, 1922.

Gosling, R., and Montanus, J.: Direct cultivation of tubercle bacilli from spinal fluids. Occurrence of human and Bovine types. J. Med. Res. 44: 513, 1924.

Griffith, A.S.: Types of tubercle bacilli in human tuberculosis. J. Path. and Bakt. 32: 813, 1929.

Griffith, A.S.: The cultural characteristics and virulence of a mammalian tubercle bacilli and the circumstances under which they vary. J. State Med. 30: 139, 1922.

Grund, M.: The reaction curve in glycerin broth as an acid in differentiating the bovine from the human type of tubercle bacillus. Journ. Med. Res. 25: 333, 1912.

Guyot, G.: Lespettorato come mezzo di cultura del bacillo della tubercolosi. Gazetta degli ospedali e delle Cliniche. 80: 1903.

Harada, K.: Antiformin or H_2SO_4 method for culture of the Tb. bacillus. Zentr. Bakt. Abt. Orig. 113: 266, 1929.

Harvey, W.F.: Bacteriology and laboratory techinique Sect. II. Indian J. Med. Res. 9: 66, 1921.

Haserodt, H.: Neue Medoden zum nachweis von Tb. bacillen insputum. Hyg. Rund schau. 19: 699, 1909.

Havas, A.: Studien uber das sputum autolysat I Mitteilung: Einfluss des Sputum Autolysats auf Wachstum, Morphologi, und Virulenz des Tuberbuloserregers. Zeitschr. Tuberk. 56: (1) 39, 1930.

Hauptmann, W. and Burtschen, J.: Diagnostic utility of isolation of tubercle bacillus according to Hohn. Wein. Klin. Wochnicher. 84, 1928.

Hawthorn: Culture homogenes du bacille de la tuberculose en eau peptones. Centr. f. Bakt. Abt. I. Ref. 33: 741, 1903.

Heise, F.H., and Brown, L.: The intracutaneaus reaction of the tuberculous to glycerin bouillon and tuberculin. Am. Rev. Tuberc. 6: 1084-86, 1923.

Henley, R.R.: The influence of iron on the growth of the tubercle bacilli. Am. Rev. Tuberc. 12: 246-59, 1925.

Henley, R.R.: The reaction and growth curves of cultures of tubercle bacilli. Rev. Tuberc. 13: 107-13, 1926.

Henley, R.R.: Dextrose in synthetic media for the tubercle bacillus. Am. Rev. Tuberc. 19: 660, 1929.

Henley, R.R., and Le Duc: Ammonia malate as a source of nitrogen for tubercle bacilli in cultures. Am. Rev. Tuberc. 22: 568, 1930.

Herman, M.: Procede rapide de coloration des bacillies tuberculeux dans les liquides et les tissues organiques. Ann. de l'Inst. Pasteur. 3: 160, 1899.

Herrmann, E.: Zur Frage der Kultur des Tuberkelbacillus und ihrer veriwendung zur Diagnose der Tuberkulose. Centr. f. Bakt. Abt. 1., Orig. 102: 169, 1927.

Herrold, Russell D.: Egg-yolk medium for the growth of tubercle bacilli. J. Infectious Diseases. 48: 236-41, 1931.

Hease, W.: Ein neues Vervalıren zur Züchtung des Tuberkelbacillus. Leitscha. f. Hyg. 31: 502-506 1899.

Hisse, W.: Methodik du Zuchtung du tuberkilbacillen aus menschlichin auswurf. Centralbl. f. Bakt. 35: 386-387, 1904.

Hoffman,: Isolation of Tb. on glycerin potato over two years. Hyg. Rundschau. 14: 305, 1904.

Hohn, S. : Four years experience with the cultivation of the tubercle bacillus for diagnostic purposes. Zentralbl. Bakt. I. Abt. Orig. 113: 366, 1929.

Hohn, : Die Kultur des Tuberkelbazillus zur Diagnose der Tuberkulose. Munchen. Med. Wochnecher 73: 2162, 1926.

Hohn, J.: Die Kultur des Tuberkelbazillus zur diagnose der Tuberkulose. Munchen, Med. Wochnecher 73: 609, 1926.

Hohn, J.: Die Kultur des Tuberkelbacillus zur Diagnose der Tuberkulose. Centralbl. f. Bakt. 98: 460, 1926.

Hov, P.C.: The cultivation of tubercle bacilli within diffusion membranes in vitro and in vivo, A preliminary report. Am. Rev. Tuberc. 17: 422-430, 1928.

Howze, H.H.: The bactericidal action of ultraviolet light on the tubercle bacillus. Am. Rev. Tuberc. 13: 470-74, 1926.

Huth, T.Von:Leiberthal, F.: Cultural demonstration of Tb. bacillus from the urine. Zeitschr. Urol. Chir. 29: 73-78, 1930.

Huth, T. von and Leiberthal,F.: The culture of Tb. bacilli from the urine. A report of 1200 cultures. Surg., Gynesol, and Obstetr. 50: 985, 1930.

Inkster,J.and Gloyne,S.R.: The bactericidal action of gastric juice on B. tuberculosis. Brit. M/J. No. 3181: 1024, 1921.

Isabolinsky, M.E. Gitowitch, W.: Ueber die gewinnung von tuberkelbazillenkulturen. Centr. f. Bakt. 94: 241, 1925.

Ishimori,K.: Uber den Einfluss der wasserstoffionen konzentration des nahrbodens auf das Wachstum der saurefesten Baktenan. Ztschr. f. Hyg. 102: 329, 1924.

Jacobi,A.: Cultivation of tubercle bacilli. Klin. Wochncher. 6: 2472, 1927.

Jensen, K.A.: Die fordand Wirkung einiger Metallsalze auf das bakterienwachetum im besonderen Hinblick auf die Zuchtung von Tuberkelbazillen. Ztech. f. Immunitatsforsch. u. exper. Therap. 46: 59, 1926.

Jobling,J.W., and Peterson, W.: Ferment Inhibiting Substances in tubercle bacilli. J. Exper. Med. 19: 251, 1914.

Jochmann, G.: Uber neuere Nährboden zur Zuchtung der Tuberkuloseerrogers, so wie uber ein neues anreicherungs verfahren hei der Untersuchung auf Tb. bacillen. Hyg. Rund. 10: 969, 1910.

Johnson, T.B., and Renfrew, A.C.: Some chemical changes accompanying the growth of tubercle bacilli on Long's synthetic medium. Am. Rev. Tuberc. 17: 508-20, 1928.

Jurewitch, W.: Kartoffelnahrbouillon zur Zuchtung der Tuberkelbacillen. Centr. f. Bakt. Orig. 47: 664, 1908.

Kahn, M.C. and Torrey, J.C.: The developmental cycle of the tubercle bacillus as revealed by single cell studies. Am. Rev. Tuberc. 18: 815-823, 1928.

Kewamura, R.: On the homogeneous cultivation of tubercle bacilli and a study with the tubercle bacillus obtained thru homogeneous cultivation. Keio-Igaku 3: 1923.

Keilty,R.A.: Study of the cultivation of the tubercle bacillus directly from the sputum by the method of Petroff. J. Exper. Med. 22: 612, 1915.

Kendall, A.T., and Day,A.A.: Studies in acid-fast bacteria. The metabolism of saprophytic human tubercle bacilli in plain dextrose, mannite, and glycerin broths. J. f. Inf. Dis. 15: 417, 1914.

Kendall,A.T., and Day,A.A.: The metabolism of certain rapidly growing human tubercle bacilli in broth free from Lipoids and fatty substances. Studies in acid-fast bacteria, II. Inf. Dis. 15: 423, 1914.

Kendall,A.T., and Day,A.A., and Walker,A.W.: The metabolism of certain rapid growing tubercle bacilli in media with inorganic salts as sources of nitrogen. Studies in acid-fast bacteria I.V.J. Inf. Dis. 15: 433-38, 1914.

Kendall,A.T., Day,A.A. and Walker,A.W.: Observations on the specificity and thermostability of the lipose developed during the growth of a rapidly growing tubercle bacillus in media of varied composition. Studies in acid-fast bacteria VIII. J.Inf.Dis. 15: 455-59, 1914.

Kendall,A.T., Day,A.A. and Walker,A.W.: The metabolism of certain rapidly growing human tubercle bacilli in a modified Uschinsky medium. Studies in acid-fast bacteria III. Jan. Inf. Dis. 15: 428, 1914.

KendallA.T., Day,A.A. and Walker,A.W.: Acomparison of the curves of Lipolytic activity and Proteolysis of certain rapidly growing human tubercle bacilli in media of varied composition. Studies in acid-fast Bacteria IX. J.I.D. 15: 460-466, 1914.

Kendall,A.T., Day,A.A. and Walker,A.W.: The metabolism of virulent human tubercle bacilli. (Studies in acid-fast Bacteria XI.) The metabolism of bovine tubercle bacilli. (Studies in acid-fast Bacteria XII.) J.I.D. 26: 45-51 to 77-84, 1920.

Kirchner,O.: Der Stoffewechsel der Tuberkelbacillus in seines Abhangigekeit von Sauerstoff und Kohlensause. Beitr. z. Klin. d. Tuberk. 68: 708, 1928.

Kitasato, S.: Reinculteren der Tuberkelbacillen. Ztschr. f. Hgy. vii. 441.

Klinimer, M.: Technik and Methodik der Pathogenen Mikroorganimen. Gustauv Fischer Jena. 409, 1912.

Kolle, W.: Wassermann,A.von: Handbuch der Technick and Methodik der Bakteriologi und Serologie. Julires Springen. 1923.

Kolmer, F.A., Davis,L.C., and Jager,R.: The influence of chalmoogra oil on the tubercle bacillus. J.I.D. 28: 265, 1921.

Kondo, Seigo: Carbohydrate metabolism of the tubercle bacillus. Biochom. Zeitr. 153: 302, 1924.

Kowalski,H.: Bakteriologische Untersuchungen uber du Influenza. Wein. Klin. Wchurschr. 3: 266, 1890.

Kuhne: New medium for cultivation of the tubercle bacillus. Ztschr. f. Biol. 30: 239, 1892.

Lange, L. and Nitsche, P.: Eine neue methods des Tb. bacillen-nachweises. Deutsch. med. Wchmschr. 35: 435, 1909.

Lanken, K.: Eppendorfer Tuberkulosebecher; Beitr. z. Klin. d. Tuberk. 62: 707, 1925.

Lanken, K.: Hundentuberkulose mit einem nichtzuchtbaren Virus. Beitr. z. Klin. d. Tuberk. 62: 709, 1926.

Larson, and Montauk: Effect of soaps on Tb. Proc. Soc. Exp. Med. and Biol. 20: 229, 1923.

Levy, E., and Blumenthal, F., Marxer, A.: Altenuation or killing of Tb. with chemically indifferent media. Centr. f. Bakt. Abt. I. Orig. 46: 278, 1908.

Lewis, P.A.: The influence of certain organic substances on the growth of the tubercle bacillus. J. Exper. Med. 25: 441, 1917.

Lichtenstein, St.: Culture of the tubercle bacillus. Centr. f. Bakt. 108: 239, 1928.

Limousin, H.: L'isolement des bacilles de Koch a partir, des crachets tuberculeux d'apris la methode de Petrof. Ann. d. l'Inst. Pasteur. 35: 558, 1921.

Lindenberg, A. and Pestana, B.R.: Chemothesapentische Versuche mit Fetten an Kulturen saurefester bacillen. Zeitschr. f. Immunitatsforsch. u. exper. Therap. 32: 66, 1921.

Lindner, P.: Neue Wege zur Bekanpfung des Tuberkelbazillus in seiner Eigenschaft als Fettpilz. Tuberculosis, 250, 1920.

Lockemann, F: Beitroge zur Biologie der Tuberkelbazillen.II. Mitteilung. Centr. f. Bakt. 69: 384, 1920.

Lockemann, G.: Beitrage zur Biologie der Tuberkelbazillen. III. Mittail. Uber den Einfluss von Lösungsstarke. Menge und Oberflachengrösse der Nahrlosurgen auf das Wschstum der Tuberkelbazillen-kulturen. Veroff. d. Robert Koch-Stiftung. 2: 105, 1921.

Lockemann, G.: Mitteil. Zuchtungsversuche mit Nahrlosungen vevscheidener Zusmmensetzung. Veroff. d. Robert Koch-Stiftung. 2: 114, 1921.

Lockemann, George: Welche Nahrstoffe sind fur des Washstum des Tuberkelbazillen unbedingt notwendy? Centr. f. Bakt. 83: 420, 1919.

Lowenstein, E.: Die Zuchtung der Tuberkelbazillen aus dem Stromenden Blute. Centr. f. Bakt. 120: 127, 1931.

Lowenstein, E.: Beitrage zur chemie des Tb. Bacillus. Centr. f. Bakt. 68: 591, 1913.

Lowenstein, E.: Uber die Reinkultur method des Tuberkelbacillus. Wein, Med. Wchvachr. 77: 767, 1927.

Lowenstein, E.: Beitrag zur Unterscheidung von offener und geschlossener Tuberkulose. Wein, Klin Wchnechr. 11: 155, 1927.

Lowenstein, E.: Beitrag zur Leistungsfahigkeit der duekten Zuchtung die Tuberkelbazillen das dem infektiosen material, mit einem Beitrag zur Geflugeltuberkulose in Menschen. Wein, Klin, Wchnechr. 37: 231, 1924.

Lowenstein, E., and Pick, E.P.: Studien uber Antigenbilding in eiweissfreien Nahmedien. I. Mitteilung Beitrage zur Kenntnis des Tuberculins. Biochem-Zeitschr. Bd. 31: 142, 1911.

Loifflen, F.: Ein neues Anreichercungs verfshren zum farberischen Nachweise sparlicher Tuberkelbacillen. Deutsch Med. Wchnschr. 36: 1987, 1910.

Lominski, I.: Nouveau milieu pour la culture des bacilles tuberculeux, pomme de terre au bouillon, a la cholesterine et a la lecithine. Soc. Biol. Varsovie. 105: 601, 1930.

Long, E.R.: A study in fundamentals of the nutrition of the tubercle bacillus: the utilization of some amino acid and ammonium salts. Am. Rev. Tuberc. 3: 87, 1919.

Long, E.R.: Chemical problems in the bacteriology of the tubercle bacillus. Am. Rev. Tuber. 5: 705-714, 1921.

Long,E.R., and Major,A.J.: A method of following reaction changes in culture of acid-fast bacteria. Am. Rev. Tuberc. 5: 715-722, 1921.

Long,E.R.: The nutrition of acid-fast bacteria. Am. Rev. Tuberc. 5: 857-69, 1922.

Long, E.R.: effect of commercial yeast vitamins on Tb. growth. (not the exact title) Am. Rev. Tuberc. 5: 705, 1922.

Long, E.R.: Chemical evidence on the phylogenetic classification of the tubercle bacillus. Am. Rev. Tuberc. 8: 195-213, 1923.

Long, E.R.: A review of some recent studies on the metabolism of the tubercle bacillus, and on the nature of tuberculin. 6: 128, 1924.

Long, E.R.: New medium for Tb. growth. J.A.M.A. 85: 650, 1925.

Long, E.R.: A non-protein medium suitable for the production of tuberculin in large quantity. Am. Rev. Tuberc. 13: 393, 1926.

Long, E.R., and Finner,L.L.: The relation of glycerol in culture media to the growth and chemical composition of tubercle bacilli. Am. Rev. Tuberc. 16: 523-29, 1927.

Luberau: Egg medium for the Tb. culture. Hyg. Rund. 17: 1455, 1907.

Lubinski, W.S.: Zur Kultiviesungs--methode Biologie, und Morphologiedèr tuberkèl bacillen. Centr. f. Bakt. 18: 125, 1895.

Lumiere,A et L.: Sur des milieux de culture solides (foie et rate) eminemment favorables au developpement des bacilles de en tuberculose. C.R.Soc.Biologie. 60: 568, 1906.

Lurie, Max B.: A comparison of the sodium hydroxide and antiformin methods for cultivating tubercle bacilli. Am. Rev. Tuberc. 7: 332-343, 1923.

Lurie, Max,B., and Kirschstein,J.: A trial of Twort's glycerol method for the isolation of tubercle bacilli. Am. Rev. Tuberc. 9: 538-543, 1924.

Lutz, Albert,: A method for transferring tubercle bacilli from solid to liquid media. Am. Rev. Tuberc. 12: 270-72, 1925.

Lyall, H.W.: The Griffith method for the direct isolation of tubercle bacilli. Am. Rev. Tuberc. 5: 899, 1922.

Malkani, M.: Die Kultur des Tuberkelbazillus (vergleichende Nahrboden untersuchung). Beitr. Klin. Tuberk. 73: 395, 1930.

Malkani, M.: The isolation of the tubercle bacillus from sputum. A comparative study of Petroff's and Corper's and Uyei's methods. Am. Rev. Tuberc. 19: 47, 1929.

Malm: Phosphorus requirement for Tb. growth. Centr. f. Bakt. 70; 141, 1913.

Manceaux, L.H.: Bacille tuberculeux et sulfate de magnesie. C.R. Soc. Biol. 91: 255, 1924.

Marpmann, F.: Uber die Herstillung unes Bakterienparates aus Kultures von Tuberkelbacillen. Centr. f. Bakt. 33: 624, 1903.

Martin, H.: Note sur la culture du bacille de la tuberculose. Archieues de medecine experimentale et d'anatomie pathologique. 1: 71, 1889.

Massol, L, et Breton, M.: Contribution de l'etude de alimentation hydrocargronee du bacille tuberculeux. C.R. Soc. de Biol. 71: 340, 1911.

Masucci, P.: A note on the effect of amiro acids on the growth of tubercle bacilli. J. Lab. Clin. Med. 6: 96, 1920.

Masur, B.: Zur Biologie des Tuberkelbacillus. Centr. f. Bakt. Abt. I. Orig. 94: 46, 1926.

Matson, R.C.: Der Vergluchesungswert einiger neueren Methoden der Sputumuntersuchung auf Tb. bacillen des Ziellschen und Muchschen types. Beitr. z. Klin. d. Tuberk. 24: 193, 1912.

Matsushita, T.: Uber die Wachstumsunterschiede des Bacillus der Menschlichen Tuberculose auf Phlenzlichen Gelatine und Agar Nahrboden. Centr. f. Bakt. 26: 125, 1899.

Matthies,T.: Culture of tubercle bacilli. Baitr. Klin Tuberk. 73: 84, 1929.

Matthies,T.: Practical work with the Isolation of tubercle bacilli by Hohn's method. Klin Wchaschr. 351, 1928.

Mayer, A. et Schaeffer,F.: Extension aucas des microbes de la notion d'acides amines indispensables. Role de l'arginine et de l'histidine dans la culture du bacille de Koch sur milieux chimiquement définis. C.R.Soc. Biol. 82: 113, 1919.

McJunkin, F.A.: Removal of acid fastness from tubercle bacilli by oleic acid or olive oil. J. I. F. 38: 520, 1926.

McJunkin, F.A.: The solvent action of oleric acid on cultures of tubercle bacilli. Am. Rev. Tuberc. 8: 393-96, 1923.

McJunkin, F.A.: Tuberculous infection in Guinea-pigs treated with tubercle bacilli made non-acid fast by incubation with olive oil. J.I.D. 38: 524, 1926.

Meissner, G. Bactericide und entwicklungshemmende Wirkung des blutes auf tuberkellbacillen. Eine neue methode zu ikrer quantitativen Messung. Centr. f. Bakt. 106: 210, 1928.

Meller, R.: Zur Nahrbodenfrage der Tuberkelbacillen.ztachr. f. Tuberk. 48: 137, 1927.

Meller,R.: Zur Branchbarkeit der Lowenstein Sumiyoshischen Reinzuchtungsverfahiens der Tuberkelbacillen fur die Diagnose. Ztchr. f. Tuber,. 44: 387, 1926.

Menzi, H.: Beitrag zur Zuchtung und zur Biologie des Tuberkel bacillus. Ztschr. f. Hyg. 39: 407.

Merrill, M.: Studies in carbon metabolism of organisms of the genus mycobacterium. J. of Bact. 21: 361, 1931.

Merrill, M.: End products of carbohydrate utilization as determined in synthetic Media cultures. J.of Bact. 21: 375, 1931.

Meyer, N.: Des Kulturelle Tuberkelbacillennachweiss in der diagnostischen Praxis. Centr. f. Bakt. 103: 345, 1927.

Mieke, Hugo: Beitragde Zur Biologie Morpholigie und Systematik des Tuberkelbacillus. Ztschr. f. Hyg. 62: 131-156, 1908-9.

Model, L.: Influence of capillary active substances on growth of tubercle bacilli. Zentr. Bakt. 113: 214, 1929.

Modern, F. and D'Alessandro, N.: Preparation of Besredka medium with egg yolk. Compt. Rend. Soc. de Biol. 99: 1427, 1928.

Moreau, E.: Application du milieu de Petroff a la culture du bacille de Koch. Rev. d.l. Tuberc. 3: 381, 1922.

Moreau, E.: Emploi du milieu de Petroff pour la recherche du bacille de Koch dans les epanchments tuberculeux et dans l'urine, Rev. d. l. Tuberc. 3: 385, 1922.

Moureau, M, et Gruvel, Ch.: Essais de culture du bacille tuberculeux dans differents extraite glycerines d' organes. Soc. Biol. Bordeaux. 1926.

Nauhaus, C.: Tuberkelbacillenzuchtung aus der Leiche,. Centr. f. Allg. Pathol. 43: 337, 1928.

Nocard and Roux: Growth of the tubercle bacillus. Ann. de l' Inst. Pasteur. 1: 19, 1887.

Novy, F.G. and Soule, M. H.: Microbic respiration. II. Respiration of the tubercle bacillus. J.I.D. 36: 169, 1925.

Nozao, S.: On the isolation of tubercle bacillus by Petroff's method. Abs. Japan Med. World. 4: 297, 1924.

Ohnawa, J.: Biological studies of tubercle bacillus. I. Communication. On glucose formation of tubercle bacillus. Zasshi, 22: 1924.

Opie, E.L.and Barker, B.I.: Enzymes of tuberculous tissue. J. Exper. Med. 10: 645, 1908.

Opie, E.L.and Barker, B.I.: Enzymes of tuberculous Exudates: J. Exper. Med. 11: 686, 1909.

Parhon, C.J.: and Squini, E.: Attempts at the cultivation of microbes on glandular media. C.R. de Soc. de Biol. 78: 161, 1915.

Park, W., and Krumweide, C.: The relative importance of the Bovine and human types of tubercle bacilli in the different forms of human Tb. J. Med. Res. 23: 205-368, 1910.

Park, W., Williams, A.W., and Krumweide, C.: Glycerol veal infusion broth. Path. Micro. 133: 1924.

Passini, F.: Uber die Lebensdauerder Tuberkulosebacillen in Kulture anaesober faulmisbakterien. Wein. Klin. Wohnschr. 38: 1182, 1925.

Pawlowsky, A.D.: Die versucheuber Tuberkulose, Material zur lehre uber dos Wachstun der Tuberkelbacillen auf verschiedenen Nahrboden und uber die Forman de Tuberculose bei verschiedenen tieren: Kaltblutern Vogeln und Saugetiernan. Zeit. f. Tuberk. 16: 1910.

Pawlowsky, A.D.: Culture des bacilles de la tuberculose sur la pomme de terre. Ann. de l'Inst. Past. 1: 302, 1888.

Petragnani, G.: Modification of my method of isolation the tubercle bacillus in pure culture from excretions and other tuberculous material. Boll. Inst. Sieroterop. 5: 173, 1926.

Petroff, S.A.: A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. Jour. of Exper. Med. 21: 38, 1915.

Petroff,S.A.: Eine neue methode zur Isolierung und Kulture des Tuberkelbacillus. Zeit. f. Tuberk. Bd. 24: 262, 1915.

Petroff,S.A.: Isolation of Tb. from sputum by new method. Bull. John Hopkins Hosp. 26: 276, 1916.

Petroff, S.A.: A glycerin extract of tubercle bacilli as an antigen in complement fixation. Am. Rev. Tuberc. 2: 523-4, 1918.

Petroff, S.A., and Dorset: Cultivation of Tb. Bacilli. Am. Med. 3: 555, 1902.

Phisaliz, M.C.: La jaune d'oeuf comme milieu de culture du microbe de la tuberculose; variability du bacille de Koch. C.A. Soc. de Biol. 55: 604, 1903.

Platonov, G.: Influence of unsaturated fatty acids on the virulence of tubercle bacilli. A.R.T. 21: 362-69, 1930.

Popper, H., and Worany, J.: Uber den tyrosin und Typtophangehalt von bouillon und Asparaigin Tuberkelbacillin. Ein Beitrag zur Frage der Cyclopoiere durch Bakt. Ztschr. f. Tuberk. 43: 368, 1925.

Porter, A.E.: The bacteriolytic action of gland extracts on tubercle bacilli. J. Hyg. XVI: 55, 1917.

Porto: The sensitiveness of the tubercle and other acid-fast bacteria to acids. J.Hyg. 26: 1918.

Proskauer, B., and Beck, M.: Beitrage sur Ernalirungs physiolo gie der Tuberkelbacillus. Ztschr. f. Hyg. 18: 128, 1894.

Raw, N.: Attenuation of tubercle bacilli extending over a period of nineteen years. J. Path. and Bakt. 29: 134, 1926.

Raw, N.: Attenuation of human, bovine and avain tubercle bacilli Lancet. 376, 1919.

Reed, G.B., and Rice, C.E.: The action of iron and cituate in synthetic media for tubercle bacilli. 16: 92, 1928.

Ritchie, W.T.: The wax of tubercle bacilli in relation to their acid resistance. J.Path. and Bakt. 10: 334, 1905.

Robinovitch, L.G., and Stiles, G.W.: A chemical basis for the treatment of tuberculosis experiments on the action of steapsin and insulin on tubercle bacilli. Am. Rev. Tuberc. 9: 587-612, 1924.

Rochais, A.: Nouveau milieux vegetal pour cultures microbienes (agar au jus de coratti) C.R. Soc. de Biol. 74: 604, 1913.

Rockwell, G.E.: The influence of carbondioxide on the growth of bacteria. J. Infect. Dis. 32: 98, 1923.

Rockwell, G.E. and Highberger, J.H.: Carbon dioxide as a factor in the growth of the tubercle bacillus and other acid-fast organisms. J. Infect. D 38: 92, 1926.

Roddy, J.A.: Medical Bacteriology. A. Blakeston's Son & Co., 46, 1917.

Roguski, Jean: Remarques sur la preparation des chachats pour la culture des bacilles de la tuberculose. C.R. Soc. de Biol. 97: 961, 1927.

Roguski, Jean: Modification in the preparation of Petroff's medium. (Modification apportee a la preparations du milieu nutrituf de Petroff) C.R.Soc. de Biol. 97: 962, 1927.

Romer, P.: Ein Beitrag zurFrage der Wachstums qeschwindigkeit des Tuberkel bacillus. Centr. Blatt. 27: 1900.

Ronzoni, G.: I prodotti bronco-polmonari come tenero di coltura del bacillo tubercolose. Gazzeta medica itabiana. 47: 1903.

Roux, G. and Rochaix, A.: Preis de Microbie et de Technique Bacteriosiopique. A.Maloine,Paris. 122, 1911.

Saelhof, C.C.: The concentration of bacteria, including tubercle bacilli, by the use of aluminum hydroxide cream. Am. Rev. Tuberc. 9: 97-98, 1924.

Sanchez, J.B.: Un procedimiento para substituir al medio de cultivo de Petroff. Bull Pasteur. 23: 622, 1925.

Sander, G.: Uber das Wachstum der tuberkelbacillen auf phlanzlichen Nährboden. Arch. f. Hyg. Bd.xvi: 38, 1892.

Sasano, K.T. and Medlar, E.M.: A study of the presence of tubercle bacilli in the bile and of the tuberculose lesions of the liver in experimental animals. Am. Rev. Tuberc. 10: 392-403, 1924.

Sauton, B.: Sur la nutrition minerale du Tb. bacilli. Compt. Rend. Acad. Sci. 155: 860, 1912.

Sauton, B.: Comparative action of Gismuth and certain antiseptics on Tb. C. R. de Soc. de Biol. 76: 66, 1914.

Sazerac,: Culture du bacille tuberculeux sur milieu a base de levure autolysee. Centr. f. Bakt. 72: 162, 1921-22.

Schiller, I.: Uber enzungene Ant-gonitem V. mitt. Centr. f. Bakt. 304, 1927.

Schiller, J.: Vorversuche zur Zuchtung der Tuberkelbacillen and sauesfesten Saprophyten im auswarfes. Centr. f. Bakt. 96: 92, 1925.

Schlossberger, H. and Prigge, R.: Versuche zur kultu rellen Diferenzierung der säurefesten Bakt. Ztsch. f. Hyg. 99: 186, 1923.

Schmidt: Uber wachstum fündende Eigenschaftes der Filtorate von Tuberkelbacillen Kulturen und andener sutoffe. Centr. f. Bakt. 94: 94, 1925.

Schmidty: Tierversuchund Kulturvenfahren zur Nachweiss von Tubkelbacillen in Sputum. Centr. f. Bakt. 101: 364, 1927.

Schmidt, F. and Sylla, A.: Zur Frage der Zuchtung von Tuberkel aus mikroskopisch negativem material. Ztschr. f. Tuberk. 45: 370, 1926.

Schmidt, H.: Uber Beziehungen zwischen Tuberkelbazillen und schimmelpilzen. Beitr. z, Klin d. Tuberk. 46: 456, 1921.

Schmurer: Uber verandsrungen säurefesten Bakterien in Kulturen auf saponinhaltigem Nährböden. Zbl. f. Bakt. 89: 5150, 1922.

Schobl, O: Chemotherapentic experiments with chaulmoogra and allied preparations. V. An inquiry into the mechanism and nature of the growth- inhibiting effect of chaulmoogra and other vegetable oils. Philippine J. Sci. 25: 135, 1924.

Schobel,O : Chemotherapentic experiments with chaulmoogra and allied preparations. VI. A survey of certain orgainic compounds as to their growth-inhibitory activity toward acid-fast bacilli in vitro. Philippine J. Sci. 25: 123, 1924.

Schobl, O.: Chemotherapeutic experiments with chaulmoogra and allied preparations. Philippines J. Sci. 23: 533, 1923.

Schrader, G.: Die Zuchtung der Tuberkelbacillus nach der Methode von Hohn. Centr. f. Bakt. 102: 163, 1927.

Schumowski, W.: Studien an auf eiweiss freien Nährböden gezüchteter Tuberkulose. Centr. f. Bakt. 26: 155, 1899.

Schurer: Über Veränderungen säurefester Bakterien in Kulturen auf saponinhaltigen Nährboden. Centr. f. Bakt. 89: 150, 1922.

Serkowski, Stanislaus: The demonstration of increased numbers of tubercle bacilli in the excretions of tuberculous patients. Am. Rev. Tuber. 8: 85-92, 1923.

Sulemann, M. and Klingmuller, H.: Die zuchtung von Tuberkelbacillen aus verschiedenem Ausgangsmaterial mit Hilfe des sogenannten Saure behandlungs verfahrens. Zentr. f. Bakt. 104: 482, 1927.

Shope, R.E.: The influence of cholesterol on Experimental tuberculosis. J. Exper. Med. 48: 321, 1928.

Shope, R.E.: The survival of the tubercle bacillus in physiological salt solution. J. Exper. Med. 44: 623, 1926.

Sickmuller, E.: Zur Kultur des Tuberkel bazillus. Deutsche Tierarzt. Wochenschr. 38: 323-325, 1930.

Siebert, C.: Zur biologie du tuberkelbacillen. Centr. f. Bakt. 51: 305, 1909.

Simmons, J. S.: The isolation and cultivation of tubercle bacilli protected from light. Am. Rev. Tuber. 8: 165-69, 1923.

Smeeton, M. A. and Rettger Leo F.: A bacteriological study of a case of clinicl tuberculosis in which the tubercle bacillus could not be demonstrated in the sputum. Am. Rev. Tuber. 9: 443-463, 1924.

Smith, A.R.: On certain reactions of the tubercle bacillus to sperm oil and its constituents. J. Path. and Bact. 20: 395, 1915-1916.

Smith, D. T. Willis, H.S. and Lewis, M.R.: The behavior of cultures of chick embryo tissue containing avian tubercle bacilli. Am. Rev. Tuberc. 6: 21-34, 1922.

Smith, E.: Bacteria in Relation to Plant Diseases. Carnagie Instl 5731. Washington D.C. 199, 1905.

Smyth, H.F.: The reactions between bacteria and animal tissues conditions of artificail cultivation. J. Exper. Med. 23: 283, 1916.

Sommenschein, C.: Isolation of tubercle bacilli according to Hohn and its practical use of bacteriological and chemical diagnosis. Beitr. z. Klin. Tb. 67: 45, 1927.

Sonnenschein, Curt: Cultural demonstration of Tb. organisms in milk by means of malachite green egg medium. Deutsch. Tierarzt Wochenschr. 38: 115, 1930.

Soparkar, M.B.: Cultivation of tubercle bacillus directly from sputum and post mortem material. Ind. f. Med. Res. 4; 28-46, 1916.

Spengler, Carl: Die Sengzuchtung der tuberkel bacillen aus Sputum. Ztschr. f. Hyg. 51: 338.

Spengler, Carl: Tuberkelbacillen zuchtung aus bakterien dermischen und formaldehyd desinfection. Ztschr. f. Hyg. 42: 91, 1903.

Stitt, E.R.: Practical Bacteriology, blood work and animal Parasitology. P. Blakeston's Sons & Co. 38, 1923.

Stoeltzner, W.: Uber versuche die Tuberkulose durch Wachalosliche staffe zu besinflussen. Cntr. f. Bakt. 72: 182, 1921-22.

Stewart, F.C.: A note on Petroff's cultural method for the isolation of tubercle bacilli from sputum and its application to the examination of milk. J. Exper. Med. 26: 755, 1917.

Suyenago, B.: An investigation of the acid fastness of tubercle bacilli II. Am. Rev. Tuberc. 4: 526-29, 1920.

Suyenago, B.: An investigation of the acid fastness of tubercle bacilli. Am. Rev. Tuberc. 13: 550-69, 1926.

Sweany, H.C.: The regeneration of acid fastness in apparently degenerated tubercle bacilli. Am. Rev. Tuberc. 18: 630-661, 1928.

Sweany, H.C.: The granular of the tubercle bacillus. Am. Rev. Tuber. 17: 53-77, 1928.

Sweany, H.C.and Evanoff, M.: The effect of sanocrysin on the tubercle bacillus aftter long exposure in vitro. Am. Rev. Tuber. 14: 523-32, 1926.

Sweany,H.C. and Evanoff, M.: Further studies on the cultivation of the tubercle baillus. Am. Rev. Tuberc. 18: 661-672, 1928.

Sweany,H.C. and Evanoff, M.: The isolation of tubercle bacilli from septic material. Am. Rev. Tuberc. 17: 47-53, 1928.

Sweany, H.C.and Evanoff M.: The value of culture in the diagnosis of tuberculosis. Tuberc. vii; 404; 1930.

Sweany, H.C. and Stadiuchenko, A.: A review of the incubation method of sputum concentration. Am. Rev. Tuberc. 22: 420, 1930.

Sweany, H.C. and Stadnichenko, A.: A system of sputum analysis for the presence of acid fast bacilli. J. Lab. and Clin. Med. 14: 547, 1929.

Sweany, H.C. and Wasick, M.M.: The effect of senocrysin on the tubercle bacillus in vitro. Am. Rev. Tuberc. 12: 316-43, 1925.

Szaboky,J.: Ein Beitrag zur keunteus du Kulturellen Eigenschafter du Tuberkel bacillen. Centr. f. Bakt. 43: 651, 1907.

Thornot,H. and Masselin,E.J.: Precise de microbie. Masson et Cie. 48, 1902.

Thompson, E. T.: Symbiotic growth of B. Protsus and B. tuberculosis, appearance of an acne-like organism. Lancet, 199: 186, 1920.

Tiffeneau, M. et Marie, A.: Sur diverses conditiond de culture du bacille tuberculeux. Bull Past. 10: 206, 1912.

Timofejewsky, A.D. and Benewolenskaja,S.W.: Zur Frage uber die Reaktion patholischer Leukocytem-formen des menshenbluts in vitro auf Tuberkellbacillen. Virchow's arch. f. Path. Anat. 264: 605, 1927.

Tomaczewski, E.: Uber das Wachstum des Tuberkelbacillen auf Kartoffelhaltegen Nährboden. Ztschr. f. Hyg. 32: 246, 1899.

Tulloch, W. J., Munro, W.T., Ross, G.R., and Dummings,W.: The bacteriological characteristics of one hundred strains of tubercle bacilli isolated from sputum. Tuber. 6: 18, 1924.

Turro, M. and Alamar, J.: Cultivation of Tb. Gacet Med. Catal. 40: 404, 1922.

Twort, C.C.: The isolation and preservation of tubercle vacilli by means of glycerine. Lancet, 203: 1221, 1922.

Tzetzu: Isolement direct sur milieu de Petroff das bacille tuberculeux provenant d'abscess froids, Presse Med. xxx: 503; 1922.

Uhlenhuth, P. and Kerstein, H.F.: Eine nene Methode zum Kulturelten und Mikroskopischen Nachweis von Tuberkelbacillen im sputum und anderem tuberculosem material. Ztschr. f. Exper. Path u. Therap. 6: 759, 1909.

Uschinsky: Synthetic medium for growth of tubercle bacillus. Centr. f. Bakt. 14: 316, 1893.

Uyei, N.: Vitamins and tubercle bacilli. 1. Do tubercle bacilli produce or contain growth promoting substances? J. I. D. 40: 425, 1927.

Uyei, N.: Vitamins and tubercle bacilli. 2. The effect of the commonly known vitamins on the growth of tubercle bacilli. J.I.D. 40: 433, 1927.

Uyei, N.: The glutathione content of animal tissues with reference to tuberculosis. J.I.D. 39: 73, 1926.

Uyei, N.: Nature of the growth promoting principle in the potato culture of tubercle bacilli. A.R.T. 22: 203-17, 1930.

Valletti, G.: New culture medium for rapid growth of TB. Policlinico S.M. 7: 1912.

Valletti, G.: Uber einen neuen Nahrboden sur sehr raschen Entwicklung des Tuberkelbacillen. Centr. f. Bakt. 68: 239, 1913.

Vasileau: Cultures homogenes du bacille tuberculeux. Centr. f. Bakt. Abt. I Ref. 36: 604, 1905.

Vaudremer, A.: Action de l'extrait d'aspergillus furimigatur sur la tuberculive. Bull Past. 11: 302, 1913.

Vaudremer, A.: Tuberculine et miliueux de culture du bacille tuberculeux. Bull. Past. 19: 437, 1921.

Vaudremer, A.: Un prodede de culture homogene rapide du bacille tuberculeux. Compt. Rend. d. Soc. Biol. 85: 1055, 1921.

Vaudremer, A.: Culture des bacilles tuberculeux en 24 heares sur gelase. Presse med. 102, 1926.

Vaudremer, A.: Cultures de baclles tuberculeux en 24 heures sur gelose. Bull Past. 24: 211, 1926.

Von Schweinitz: synthetic medium for cultivation for Tb. Centr. f. Bakt. 14: 330, 1893.

Webb, G.B. Ryder, C.T. and Gilbert, G.B.: The survival and virulence of tubercle bacilli in excised animal lymph nodes. Am. Rev. Tuberc. 5: 388-96, 1921.

Webb, G.B. Boissenain, C.H. and Ryder, C.T.: Gas requirements of the tubercle bacillus. Am. Rev. Tuberc. 9: 534-37, 1924.

Webb, G.B. Ryder, C.T. and Gilbert, G.B.: The attenuation of tubercle bacilli in incubated tuberculous tissue. Am. Rev. Tuberc. 12: 242-245, 1925.

Weinzirl, J. and Knapton, F.: The biology of the tubercle bacillus. 1. Hydrogen-ion concentration produced by some members of the genus of mycobaterium. Am. Rev. Tuberc. 15: 380-388, 1927.

Weise, K.: Eigelbwasser zur Zuchtung von Tuberkelbacillen. Deutsche Med. Wchnschr. 51: 270, 1925.

Weise, K.: Egg yolk fluid for isolation of Tb. out of ascites and liquor. Centr. f. Bakt. 94: 35, 1925.

Wellmann, C.: a new and efficient medium for the isolation and growth of strictly parasitic or feebly saprophytic Bacteria. Centr. f. Bakt. 66: 142-43, 1912.

Wellmann, C.: New and efficient medium or isolation and growth of strictly parasitic or faintly saprophytic Bacteria. Centr. f. Bakt. Orig. Abt. I. 66: 142.

Wherry, W.B.: Some factors affecting acid-proofness and non-acid proofness in a saprophytic culture of B. Tuberculosis. J.I.D. 13: 144-154, 1913.

Wherry, W.B. and Ervin, D.M.: The necessity of CO_2 for the growth of B. tuberculosis. J.I.D. 22: 194, 1918.

White, M. C. and Gammon, S. M.: Relation of animal fat to Tb. fat. Joun. of Med. Res. 26: 257, 1912.

Williams, W.K. and Burdick, W.: A new culture medium for the tubercle bacillus. J. Bact. I: 411, 1916.

Wilson, G.H.: On the direct cultivation of tubercle bacilli from tissues. Brit. Med. J. 146, .

Yabe, T., Shibato, M., Kumagaya, Y., and Kobayaski, K.: Studies on Yabe's strain of tuberculosis "T.Y." together with its biological nature, Abst. Japan Med. World. 162: 1925.

Zahn: Ein neues einfaches Anreicherungsver-fahren fur Tuberkelbacillen. Munchen Med. Wchnschr. 57: 840, 1910.

Ziehl, F.: Zur Fahbung der Tuberkelbacillus. Deutsche Med. Wchnschr. 8: 451, 1882.

Zlatoguroff, S., Zechnowitzer, M. and Korchkin, M.: Untersuchungen uber die Moglichkeit eines uberganges non saurefesten saprophyten in echte Tuberkelbacillen. Ztschr. f. Hyg. 105: 583, 1926.