TRANSLATION NO. 329

DATE: July 1968

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DEPARTMENT OF THE ARMY
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THE PRODUCTION OF RADIOACTIVE ANTHRAX BACILLI

(First Report)

Izvestiya Akademii Nauk Armyanskoy SSR,
Biologicheskiye i Sol'skokhozyaystvonnyye Nauki,
[Herald of the Academy of Sciences Biological and Agricultural Nauki, Armenian SSR]
Vol XII, No 1, 1959, Yerevan
Pages 69-73
Russian, per.

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In recent years, radioactive isotopes have been widely used in biology, medicine, and veterinary medicine. Isotopes as indicators are utilized to study the mechanism of metabolic processes in an organism.

The purpose of the present work was to establish the conditions necessary for the production of highly-radioactive anthrax bacilli.

Below are described certain results of the work carried out by us: a) in the search for a specific nutrient media for culturing Bac. antracis; b) in the determination of laws governing the absorption of radio-sulfur by anthrax bacilli in relation to various concentrations of radioactive sulfur in the medium; and c) in obtaining maximal growth of the culture and increasing the absorbability of radio-sulfur by the anthrax bacilli.

In the production of radioactive bacilli tracer sulfur in the form of methionine was employed.

Considering the fact that in culturing bacteria on normal nutrient media containing non-radioactive sulfur, following the addition of radioactive sulfur the absorption of the latter by the microorganisms from the medium is very insignificant, in order to obtain microorganisms with the greatest radioactivity, it was necessary to seek a nutrient medium which either did not contain sulfur or contained a minimum amount of it. Various synthetic nutrient media were tested for this purpose.

At the beginning of our investigations, a study was made of the growth of Bac. anthracis on two synthetic media: the Strogov medium and the L'vov medium. The L'vov medium contains magnesium sulfate and in order that radioactive sulfur be the only source for the microorganisms, magnesium chloride was introduced into the medium instead of magnesium sulfate.

In both synthetic media the pH was initially 7.2. The indicated media were poured into test tubes and slanted. Standard meat peptone agar was used as a control. The cultures were grown in a thermostat at 36-37°C. Confirmation of growth was carried out after 24 and 48 hours.

The growth of the anthrax bacilli on the synthetic media both after 24 hours and after 48 hours was sparse or very sparse, the concentration of microorganisms in 1 ml as compared to the culture grown on meat peptone agar being 8-10 times less. The two synthetic media mentioned therefore proved to be unsuitable for our work. We rejected the use of meat peptone agar because the presence of organic sulfur compounds can not be excluded in such a medium.

Next, we turned to a medium with another composition: 1 g of peptone, 4 ml of glycerin, 0.5 g of sodium chloride, 2.5 g of agar-agar, and 100 ml of distilled water. Such a medium was chosen because peptone contains a large amount of high molecular products of protein decomposition and is well utilized by the microorganisms and the glycerin is included as a source of carbon and energy for the microorganisms.

A comparative investigation of the glycerin-peptone and meat peptone agar for the growth of anthrax bacilli indicated that the most suitable medium for growing Bac. anthracis was the glycerin-peptone agar.

In our further investigations, the problem of the degree of absorption of radioactive sulfur by the anthrax bacilli, i.e., what percent of the radio-sulfur introduced into the medium transferred into the microorganisms, was of great interest.

Tagged anthrax bacilli were obtained by growing Bac. anthracis (Strain No 574) in test tubes on slant glycerin-peptone agar (pH, 7.2-7.4) to which different amounts of a radioactive sulfur in the form of methionine had been added.

After the culture had been kept in the thermostat for one day, the microorganisms were washed from the agar with physiological solution and centrifuged 5 times in order to eliminate the radioactive sulfur adsorbed on the microorganisms. Following the centrifuging the concentration of

microorganisms in 1 ml was determined according to a turbidity standard and the radioactivity of this 1 ml suspension was established with the aid of a surface counter.

The results obtained by growing anthrax bacilli on glycerin-peptone agar containing various amounts of radio-sulfur are presented in Table 1.

Table 1

No of Microcuries in 1 ml of the Nutrient Medium	Obtained in 1 ml of Nutrient Medium		Total Percent of Radio-sulfur Absorption by the Bacilli
	Bacteria in Billions	Microcuries in the Bacteria	
2.75	850	0.026	0.9
5.5	900	0.114	2.1
11	800	0.148	1.3
22	700	0.152	0.7

From Table 1, it can be seen that with a two and four fold increase in the concentration of radio-sulfur in the medium, the total percent of the radioactive substance absorbed did not increase proportionately. The results of our experiment show that following the addition of 5.5 microcuries of radio-sulfur to 1 ml of nutrient medium, the total percent of absorption was equal to 2.1; following a decrease in the concentration of tagged sulfate in the medium, the absorption of the radioisotope by the microorganisms decreased (0.9%); and following its increase, the percent of absorption was also reduced (1.3-0.7). In the given instance, the optimal concentration of radioactive sulfur was 5.5 microcuries per ml of nutrient medium. This amount of radio-sulfur was utilized by us from then on in producing tagged anthrax bacilli.

Thus, in examining these data, no proportional, regular, simultaneous increase in the concentration of the radioactive substance in the medium, nor increase in the percent of absorption of radio-sulfur by the bacteria, were observed.

It should be noted that in removing the radioactive sulfur adsorbed on the microorganisms by centrifuging, the first and second washes revealed rather high radioactivity while the last washing (the fifth)

yieldod a significant roduction in radioactivity and the wash water showod only slight radioactivity. In addition, a separato oxperiment was performed to study the radioactivity of the anthrax bacilli after various periods of growth (after 24 and 48 hours). As a result of the experiment conducted, we found no special difference in the radioactivity of the anthrax bacilli in a 1 ml suspension containing a 1 billion concentration following culturing for 24 and 48 hours.

In further investigations we attempted to increase the absorption of radio-sulfur by the anthrax bacilli. M. Ya. Korn (6) in producing tagged dysentery bacteria and N. P. Plotnikov (7) in culturing brucella on radioactive nutrient media noted that the degree of absorption of radio-sulfur was closely connected with the intensity of the growth of the microorganisms.

It was necessary for us to seek substances which, upon addition to the nutrient medium might noticeably intensify the reproduction of the microorganisms. We found vitamins to be such substances. The importance of vitamins to the vital activity of microorganisms is tremendous. It is well known that many vitamins are synthesized by microorganisms, but it is another group of microorganisms which is required for the preparation of vitamins.

Vitamin B_6 (Pyridoxine) is necessary for the growth of the roots of certain plants, and also for the vitality of some bacteria, yeasts, and mold fungi, for which it is a growth factor.

According to the data of V. A. Devyatinin (3), lactic fermentation bacteria development extremely slowly and meagerly on media which do not contain pyridoxine. The addition of this vitamin, even in dilutions of 0.00005%, exerts a positive effect on the growth of the bacteria. The author cites similar reports from other sources which state that various B vitamins, among them pyridoxine, when used in nutrient media have an acutely manifested effect on the growth of staphylococci.

V. N. Bukin and his co-workers (1), note that the original synthesis of B_{12} was accomplished naturally by microorganisms. Echerichia coli is sensitive to vitamin B_{12}. Taking this characteristic of Bact. coli into account, this microbe is utilized for the determination of vitamin B_{12}, i.e., by a microbiological method.

R. D. Gol'tsova (2), while studying the effect of vitamins on biosynthetic processes in yeast grown on radioactive nutrient media, observed that the absorption of tagged sulfate by the yeast cells increased following the addition of certain vitamins (pyridoxine, biotin, thiamine, and others) to the nutrient media.

Table 2

Absorption of Radioactive Sulfur by Anthrax Bacilli Grown on Nutrient Media Containing Vitamins

Nutrient Media With Vitamins	Number of Microcuries in 1 ml of Nutrient Medium	Obtained in 1 ml of Nutrient Medium		Total Percent of Radio-sulfur Absorption by Bacilli	Percent of Increase in Radio-sulfur Absorption
		Bacteria in Billions	Microcuries in the Bacteria		
Vitamin B_6	5.5	1.1	0.152	2.8	140
Vitamin B_{12}	5.5	1.2	0.165	3.0	150
Vitamins B_6 and B_{12}	5.5	1.4	0.195	3.6	180
Without Vitamins	5.5	0.8	0.110	2.0	100

Conclusions

1. We succeeded in preparing a nutrient medium which did not contain sulfur for the cultivation of anthrax bacilli. This medium is a glycerin-peptone agar.

2. Following the culturing of Bac anthracis on the glycerin-peptone agar containing radioactive sulfur in the form of methionine, the bacilli were tagged since they had absorbed the radio-sulfur.

3. Following an increase in the concentration of radioactive material, no proportional regularity was noted in regard to the increase in the total percent of absorption of radio-sulfur by the bacilli.

4. The optimal concentration of radio-sulfur in the nutrient medium for culturing Bac. anthracis was 5.5 microcuries per ml of medium.

5. Following the addition of vitamin B_6 to the nutrient medium containing radioactive sulfur, the growth of the microorganisms increases and their radioactivity goes up correspondingly by 40%. Following the addition of vitamin B_{12}, absorption of the sulfate increases by 50%.

Following simultaneous addition of vitamins B_6 and B_{12} to the nutrient medium the radioactivity of the anthrax bacilli increases, as compared to a medium without vitamins, by 80%.

Submitted 6 February 1958

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