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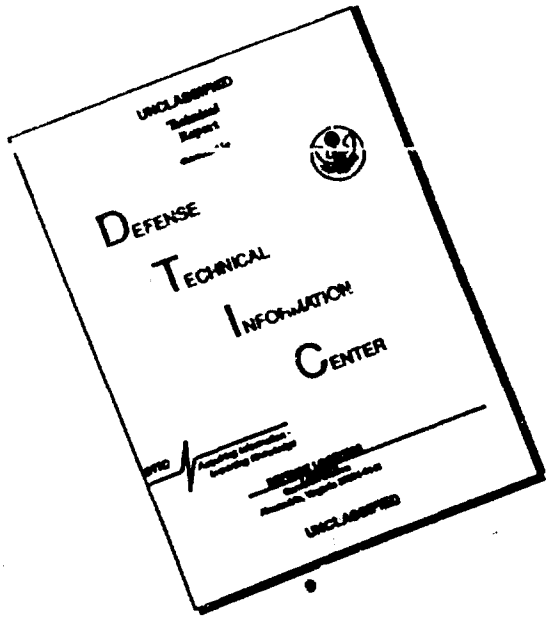

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A STUDY OF CONDITIONS OF CULTIVATION OF THE YE V VACCINE BY THE DEPTH METHOD

By
N. I. Nikolayev, Ye. V. Chibrikova, L. P. Pavlova, M. V. Altukhov, Z. A. Obukhova, Ye. E. Bakhrakh, Ye. I. Korobkova, I. V. Liskina, B. Yu. Petrova, A. N. Kraynova, N. K. Murav'yeva, A. F. Filippov, and F. K. Drozdovskaya.
(Saratov)
pages 260-265.

One of the methods of cultivation of microbes in the preparation of vaccines is the depth method of bacterial cultivation with continuous aeration and mechanical mixing. However, even here we have an insufficiently high yield of the microbe mass per one unit of volume of the culture medium.

Therefore, when we posed the problem of a detailed development of the rules of production of the antiplague vaccine, we first of all strove to determine the optimum conditions of cultivating of the YeV vaccine strain, which would assure the maximum yield of the microbe mass with a high percentage of living cells. With this in view we conducted experiments on the study of the effect produced by the buffering of the nutrient medium and addition of glucose to it, on the yield of the microbe mass.

As the nutrient medium we used broth from the fermentation meat hydrolyzate according to Huttinger, containing 190-200 mg% of amine nitrogen, with pH = 7.2.

The culture was grown at 26-28°C in reaction tanks with the capacity of 500 liters, containing 150 liters of broth. The aeration was achieved by the delivery of sterile air (0.5 liters per 1 liter of medium per 1 minute) and mechanical mixing at the rate of 360 rpm. In conformity with the problems posed, the cultivation time in individual experiments continued from 24 to 72 hours.

The inoculum was the 22-hour culture of the YeV strain, grown in Huttinger's boullion under aeration conditions. The inoculation dose was not less than 300 million microbe bodies (m. b.) per 1 milliliter of the culture medium, according to

the GKI [Gosudarstvennyy Kontrol'nyy Institut Meditsinskikh Biologicheskikh Preparatov imeni L. A. Tarasevicha; State Control Institute of Medical Biological Preparations imeni L. A. Tarasevich -- SCI] standard of cloudiness.

In the process of cultivation, every three hours samples were taken from the reaction tank, in which the optical and biological concentrations of microbes and pH of the microbe suspension were determined. Moreover, each sample was checked for the net growth by the bacterioscopic and bacteriological methods.

The biological concentration of the microbe suspension was determined by the inoculation of specific solutions of the suspension on agar plates with the subsequent count of the colonies grown. The percentage of living cells was computed with respect to the ratio of grown colonies to total number of microbes inoculated, the count of inoculated microbes being determined by the SCI standard of cloudiness.

We know that in cultivating the plague microbe in a culture fluid, the reaction of the latter changes toward the alkali, up to pH 8.0-8.2 and higher, which creates unfavorable conditions for the propagation of the culture. Therefore, in the first series of experiments we studied the effect on the growth of YeV vaccine strain, produced by the addition to the culture medium of the phosphate buffer pH = 7.2 (M/50-M/60 of one mole) in order to maintain the reaction of the culture medium in the process of microbe cultivation at the level optimal for the growth of the plague microbes (pH 7.2-7.3). This should contribute to a greater yield of the microbe mass.

It transpired, that the addition to the culture medium of phosphates in the concentrations mentioned above, did not create the necessary buffering of the nutrient medium. As a rule, in the process of cultivation of the plague microbes, the pH of the culture medium also increased at the same rate as in the control without the buffer. In these instances the optical concentration of microbes in the nutrient medium without the buffer and with it, was the same. However, if the buffer added to the culture medium, retained somewhat the pH of the culture medium in the process of cultivation, then the final optical concentration of microbes in the culture medium with the buffer was higher than in the culture medium without the buffer (9 and 4 billion, respectively, Fig. 1). The per cent content of living microbes remained high in the course of the entire experiment in both media.

In the adduced experiments the cultivation continued 24 hours and the stationary phase of growth did not occur. Therefore, in the next experiment the time of cultivation was lengthened to 36 hours.

In this instance the stationary phase occurred in 24 hours, with the 8 billion/milliliter optical concentration of

microbes in the suspension; after 30 hours the partial lysis of microbes began and in 36 hours in 1 ml of the suspension 7 billion microbes were present. The count of living microbes in the slurry attained the highest level, namely 104%, by 27 hours of cultivation (the paradoxical nature of this figure will be explained later), then it decreased and after 36 hours was 21%.

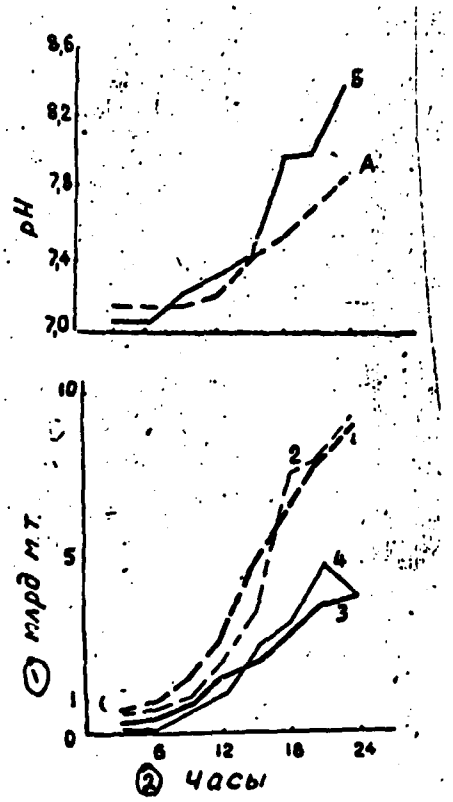

Fig. 1. The Optical and Biological Concentrations of Microbes and pH of the Culture Medium When Cultivating the YeV Culture in Huttinger's Boullion With and Without the Buffer.
In the boullion with the buffer: 1) optical concentration; 2) biological concentration; A) pH of the culture medium. In the boullion without the buffer: 3) optical concentration; 4) biological concentration; B) pH of the culture medium.
LEGEND: (1) billion microbe bodies (m.b.); (2) hours.

In this experiment after 18 hours of cultivation a portion of the reaction culture was poured off and the settled down sediment of the microbe cells was desiccated

by the lyophilization method. The living microbe count in it before drying was 84.8%, after drying, 37.6% (with the optical concentration of 70 billion/milliliter microbes). Here it was noted that in all experiments the microbes grown in the culture medium with the buffer, precipitated faster and more intensively that in the microbe suspension on the unbuffered culture medium.

The experiments performed enable us to conclude that the maintenance of pH of the culture grown at the level, near the optimal, contributes to the increase of a well-precipitating microbe mass, with a large per cent count of living cells, the amount of which remains at a high level even in a desiccated culture.

However, as we noted earlier, upon addition to the culture of a buffer in the 1/50-1/60 mole concentration, as a rule, the necessary buffering of the culture medium does not take place. On the other hand, an increase of the phosphate concentration to M/30-M/15 has an unfavorable effect on the growth of the plague microbes, which was verified by special experiments made under laboratory conditions.

Therefore, in subsequent experiments on the exposition of the effect of glucose on the yield of microbe cells under industrial conditions, the former was also used for the control of the pH of the medium.

The glucose was introduced into the nutrient medium in small doses with short intervals (the drop method), or in larger doses with longer time intervals (intermittent addition of glucose).

Glucose in the form of a 40% sterile solution was added into the reaction tank after the pH of the medium attained the 7.4 level. If the addition of glucose caused a decrease of the pH of the nutrient medium to 7.2-7.0, the addition of glucose was stopped until the reaction became again more alkaline. By the end of the experiment, with the considerable alkalization of the nutrient medium, the amount of the added glucose was increased.

In Fig. 2 we adduce the results of experiment, in which the plague microbe culture (the YeV strain) was grown 48 hours in reaction tanks under similar conditions, with the exception that in one reaction tank glucose was added (intermittently, with one-hour intervals), and in the other tank glucose was not added. A total of 0.5% glucose of the volume of the culture grown, was added. From the picture we see that the addition of glucose enables us to have a considerably larger yield of the microbe mass (19 billion per 1 milliliter) as compared with the culture medium in which cultivation was done without any additional nutrition (9 billion per 1 milliliter).

In the next experiment, in which microbe cultivation continued also 48 hours, the values of multiplicity factor

and the dose of glucose injected were determined. Glucose was added into two reaction tanks, into one -- every hour, and into the other -- with 3-hour intervals. The amount of the glucose added was controlled depending on the pH level of the culture medium, striving to keep it from changing toward the alkaline and at the same time not let it decrease below the optimum for the plague microbe cultivation. In this experiment approximately 0.75% of dry glucose was added (in introduction at 3-hour intervals) and 1% (when introducing it with 1-hour intervals) with respect to the volume of the culture grown. The optical concentration of microbes in the reaction tank, where the additional nutrition with glucose was done every hour, was 30 billion per 1 milliliter; in the other reaction tank, with the additional nutrition every 3 hours, -- 27 billion per 1 milliliter.

In the last experiment in which microbe cultivation continued 72 hours, glucose was added also in both reaction tanks, but into one it was intermittently with 1 hour intervals, and into the other, by the drop method. By the drop method 1.15% of glucose was added, by the discontinuous method, -- 1%.

The highest optical concentration of the grown culture was approximately the same in both instances -- 26 billion m. b. with drop addition of glucose and 28 billion m. b. with discontinuous addition of nutrition with 1-hour intervals (Fig. 3). Here the biological concentration of microbes in the culture, as well as in the preceding experiments with the use of glucose additional nutrition, was maintained at a high level. A good precipitation of bacteria in the suspensions was noted, which were obtained in cultivation with additional nutrition with glucose, -- after 1 or 2 hours of settling of the samples the liquid over the sediment was almost completely clear.

In growing the culture with the additional glucose nutrition there was a lengthening of the logarithm phase of the culture growth, which the curves in Figs. 2 and 3 indicate. In one instance the stationary phase occurred after 45 hours, in the remaining instances the optical concentration of microbes continued to increase during the entire observation time. In the last experiment the stationary phase was manifest only after 66 hours of cultivation.

The biological concentration in all experiments with the additional glucose nutrition increased with certain fluctuations parallel to the increase of the total microbe concentration (See Figs. 2 and 3). The highest count of living microbes was observed in the samples taken 30-48 hours after the beginning of cultivation.

Almost in all samples taken after 30-36 hours of cultivation, the percentage of living microbes was very high, approximately 100% and even higher (120-140%). Upon the

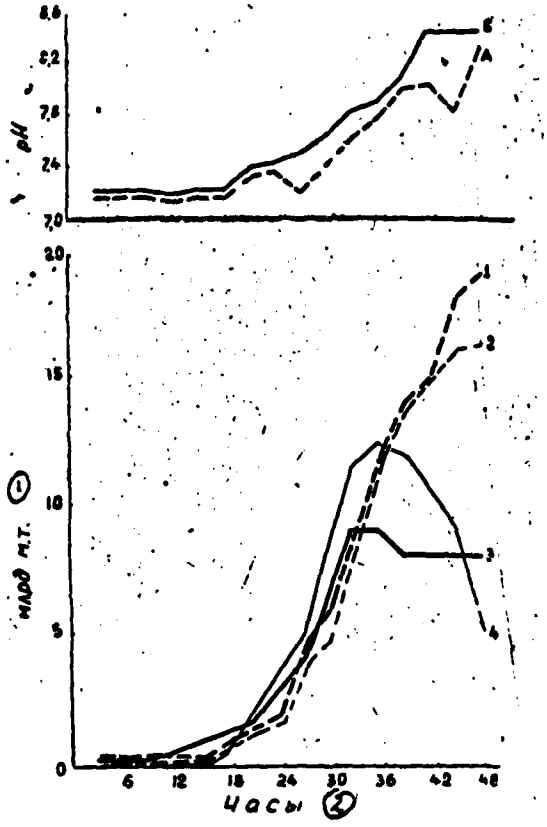

Fig. 2. Optical and Biological Concentrations of Microbes and pH of the Cultural Medium in Cultivating YeV in Huttinger's Boullion With Additional Glucose Nutrition and Without It.

In the Boullion With the Additional Glucose Nutrition of the Culture: 1 -- Optical concentration; 2 -- biological concentration; A - pH of the culture medium. Without Additional Glucose Nutrition of the Culture: 3 -- optical concentration; 4 - biological concentration; B -- pH of the medium.
LEGEND: (1) billion m.b. (2) Hours.

examination of smears from these samples it was noted that the microbe cells were smaller than in the preceding samples. Determining the microbe concentration in these samples according to the SCI standard of cloudiness for general purposes, we unavoidably lowered the true microbe count, since with the same degree of cloudiness there is always more smaller microbes in the slurry, than there is larger microbes (Fikhman, 1960).

The result of this was the increased percentage of living microbes in the slurry, inasmuch as the percentage of living microbes is the number of grown colonies/total number of inoculated microbe ratio. We may assume that were the microbe concentration determined in the hours of growth of the microbe population according to the specific cloudiness standard, the above-mentioned paradox (120-140% of living cells) would not be observed. The microbe count by another method (according to Right) showed the correctness of this assumption.

In the experiments in which glucose was added to the growing culture by various methods, a portion of the culture was poured off in a day or two. The microbe precipitate obtained after settling in the cold, was desiccated by the lyophilization method according to the rule indicated in the acting instruction (1961).

The results of determination of living microbes in these series of experimental vaccine before and after desiccation, adduced in Table 1, have shown that before desiccation the microbe slurry obtained in cultivation with the glucose additional nutrition, contained a high percentage of living cells. The dying off of microbes in the process of drying occurred within the limits admissible by the instruction, i.e. it did not exceed 50%.

A higher percentage of living cells in two-day old cultures as compared with the one-day old culture shows that with the additional nutrition with glucose, in connection with the lengthening of the logarithmic phase of the growth, the maximum yield of living microbes is attained at a later time.

On the basis of the above-mentioned data we may draw the conclusion that the cultivation of the YeV vaccine strain in Huttinger's boullion with the phosphate buffer (M/60) and additional nutrition with glucose in the process of growth of the culture, enables us to obtain thick, well-precipitating microbe suspensions, which contain a high percentage of living microbes.

Upon the addition of glucose by the drop method or in small portions with short intervals the sharp fluctuation of pH of the culture in the process of cultivation is eliminated and thereby more stable and favorable conditions are created for bacterial cultivation.

For the nutrient medium with the phosphate buffer (M/60) the optimum time of cultivation of the YeV strain, which assures the greatest yield of the microbe mass with a high percentage of living bacteria, is in the range of 24-27 hours. In growing on the same culture medium with the additional nutrition with glucose, by virtue of a considerable lengthening of the logarithmic growth phase, the optimum cultivation time is from 36 to 42 hours.

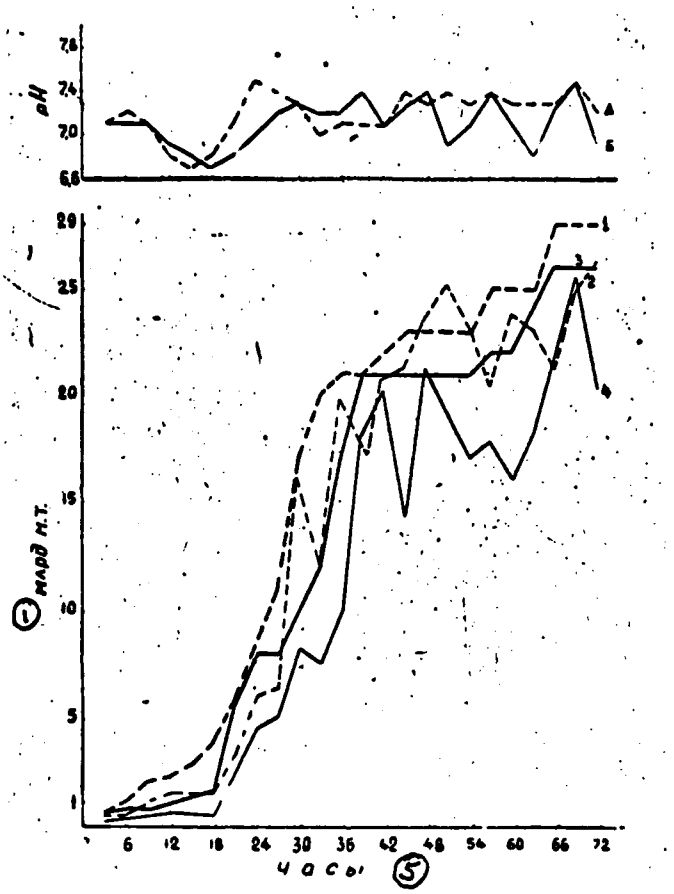

Fig. 3. Optical and Biological Concentrations of Microbes and pH of the Culture Medium in Cultivating YeV in Huttinger's Boullion With the Additional Nutrition With Glucose Intermittently With One-Hour Intervals and by the Drop Method.

With the additional glucose feeding of the culture medium intermittently: 1 -- Optical concentration; 2 -- biological concentration; A -- pH of the culture medium. With the additional glucose nutrition of the culture medium by the drop method: 3 -- optical concentration; 4 -- biological concentration; B -- pH of the culture medium.

LEGEND: (1) billion microbe bodies; (2) hours.

Table 1.

The Living Microbe Count of Experimental Vaccine.

(1) Возраст реакторной культуры (сутки)	(2) Интервал между добавлениями глюкозы, часы	(3) % живых микробов	
		(4) до сушки	(5) после сушки
1	1	40,5	17,6
	3	44	22,1
	1	65	45,5
	(6) капельный	58,6	35,5
2	1	65	45,5
	3	67,3	48,3
	1	100	49,1
	(6) капельный	95	66,1

LEGEND: (1) Age of the reaction tank culture (days); (2) Interval between the additions of glucose, hours; (3) % of living microbes; (4) before desiccation; (5) after desiccation; (6) drop method.

In the cultures of YeV strain, grown in Huttinger's boullion by the depth method, the optical concentration of microbes fluctuates in the range of 3-9 billion microbe bodies per 1 milliliter with the living microbe content from 60 to 90%.

The experiments ~~conducted~~ by us showed that on the same Huttinger's culture medium and in the same reaction tanks in cultivating YeV by the depth method with the additional nutrition with glucose, we can have cultures with the optical concentration of 19-30 billion microbe bodies per 1 milliliter with the living microbe content from 50 to 95%, which increases approximately 3 times the yield of the microbe mass.

BIBLIOGRAPHY

Instruktsiya po tekhnologii proizvodstva protivochumnoy zhivoy sukhoy vaktsiny (Instruction on the Technology of Production of Anti-Plague Living Dry Vaccine). Approved by the Assistant Minister of Health USSR, M. Ya. Nikitin, 21 June 1961. No. 370-61.

Fikhman B. A. Opticheskaya standartizatsiya bakteriynykh preparatov (Optical Standardization of Bacterial Preparations), the Bureau of Scientific Information, Moscow, 1960.