MICROBIOLOGICAL DETERMINATION OF DEOXYRIBONUCLEIC ACID

Titrimetric Estimation of Thymidine

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One of the most sensitive and specific methods for quantitative estimation of deoxyribonucleic acid (DNA) is the microbiological one developed by Hoff-Jørgensen (1951, 1954). This method is based on the fact that Thermobacterium acidophilum (Lactobacillus acidophilus) strain R 26 Orla Jensen is unable to grow in the absence of deoxyribosides. The DNA containing sample is digested with deoxyribonuclease, and a portion is added to a medium which is complete, except for deoxyribosides. The chemically defined medium of Lövtrup and Roos (1957) may be used. After inoculation and incubation the growth response is estimated turbidimetrically. About 1 μg of DNA is needed for this method. If further sensitivity is required, the response may be determined in a different way. It seems that the method worked out by Lowry and Bessey (1944) for microdetermination of riboflavin could be useful. In this method, the growth of the bacteria is estimated by microtitration of the lactic acid produced by the cells. The present paper contains a description of a method for determination of thymidine (which is taken to represent all deoxyribosides) in which the principles of Lowry and Bessey have been adapted for use with our bacterial strain. It will be shown that it has been possible in this modification to increase the sensitivity of the method about 500 times. The treatment of the DNA samples in such small quantities also requires a special technique, but this question will be dealt with in a separate paper.

METHOD

Except for the changes described here, the microbiological technique follows closely that described by Hoff-Jørgensen (1951, 1954). The double strength basal medium described by Lövtrup and Roos (1957) was found to give rather high blank values, and it was therefore found expedient to halve the concentrations of all components except the vitamins. For the thymidine standard curve the stock solution (1 mm in 25 per cent ethanol, i. e., 24.2 mg per 100 ml) is diluted 5000 times (100 μL to 500 ml), the resulting concentration thus being 0.2 μM. This solution is used undiluted and in the dilutions 0.75, 0.50, 0.25, and 0.125 for the standard curve. The whole procedure is carried out in pyrex tubes of the type used for microtitrations (Linderström-Lang and Holter, 1940). During incubation the tubes are covered with aluminum capsules (figure 1). As it has been found

Figure 1. Titration tube with aluminum capsule

inexpedient to autoclave the tubes after addition of the medium and thymidine, the tubes are placed in an aluminum block holder and sterilized at 110 C for 2 hr. The medium and the thymidine solutions are autoclaved and carefully added with constriction pipettes (30 to 40 μL) made to deliver at constant pressure. Precautions are taken to do the pipettings as aseptically as possible, thus the person carrying out the operations wears a mask, and the pipettes, etc., are autoclaved. It is not possible to inoculate in the
usual way; instead the bacteria are added to the medium before this is transferred to the microtubes (Lowry and Bessey, 1944). As will be discussed below, the concentration of bacteria is crucial for the shape of the standard curves. A suitable inoculation is obtained in the following way: A 5-ml inoculum (Hoff-Jørgensen, 1951, 1954; Løvtrup and Roos, 1957) is centrifuged, the bacteria are resuspended in 10 ml of 0.65 per cent saline; of this 2 drops are transferred to a new portion of 10 ml saline, and from this 1 drop is added to 2 ml medium. This solution, as well as the thymidine dilutions, are transferred to small beakers, from which the pipettes are filled. For each determination 4 tubes are used. The tubes are incubated at 37 C for about 44 hr. Before titration, indicator is added (10 µL of 0.04 per cent bromthymol blue). The titration is carried out with 0.1 N NaOH diluted with indicator (150 ml NaOH + 25 ml indicator). The stirring is performed by an electromagnetic “flea” according to the method described by Linderstrøm-Lang and Holter (1940). For the titration end point an intermediate green color is chosen. It is very important that the light source is good. We have found that the daylight device developed by Richter and Barfod (1948) serves this purpose excellently. In our laboratory we have constructed an apparatus in which the electromagnet and light source are combined in one unit (figure 2). The burette is a modification of the one devised by Heatley (1935). There is
obtained times. The distilled water. The tubes concentrated of the can readings the diameter thus, uL, raising in titration. It is made used to the and used out with the holder when filled and used to force the meniscus. The thymidine concentration of 1.0 contains about 48 μg per μL; our highest dilution, 0.125, thus about 6 μg per μL. In the incubation mixture the concentration is 3 μg per μL. As we add 30 to 40 μL of thymidine solution in each tube, the smallest amount per tube is seen to be around 200 μg. If the sensitivity of the method is to be increased further, it will be necessary to reduce the volumes. It would probably be feasible to decrease the volumes in the present method 3 to 4 times, but if still higher sensitivity is wanted, it seems that other methods to estimate the bacterial growth will have to be employed.

As mentioned above, the concentration of the inoculum is of importance for the shape of the standard curve. In figure 4 are shown standard curves obtained when the inoculum is 2.5 times more diluted or concentrated, respectively. The age of the milk cultures from which the inoculum tubes are obtained is also of influence. Routinely, we therefore use 1-day-old milk cultures. The incubation time also influences the shape of the standard curves. In figure 5 are shown curves obtained after 22 and 68 hr. After 22 hr the lactic acid production is lower than after 44 hr. Prolonged incubation improves the sensitivity, but the shape of the standard curve is no longer linear.

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SUMMARY

A microbiological method for estimation of deoxyribosides has been developed, in which as
DETERMINATION OF DEOXYRIBONUCLEIC ACID

little as 200 μg can be determined in one titration. In order to obtain reasonable accuracy, about 1000 μg is the minimal amount which can be determined with the method.

REFERENCES


