Arch. Inst. Razi, 1979,31, 55-59

STUDIES ON THE MODIFICATION OF DORSET - HENLY MEDIUM FOR THE PRODUCTION OF BOVINE TUBERCULIN.

By:

Hedayati, H. Alé-Agha, S. Mahinpoor, M. and Sadri, M.

Introduction:

A synthetic medium was first introduced by Dorset (1) and used for the production of tuberculin. Few years later a slight modification was made by Henley (2). Because of its world wide application it has been subjected to many changes with regard to its ingredients (7). The following formula has been currently used in most laboratories (5) for production of Bovine tuberculin.

1)	L-Asparagine	14	gr:
2)	Monopotassium phosphate anhydrous	1.5	»
3)	Magnesium sulphate MgS04, 7H20	1.5	»
4)	Sodium citrate Na3C6H507, 2H20	0.74	»
5)	Zinc sulphate ZnS04, 7H20	0.08	»
6)	Ferric citrate FeC6H507, 5H20	0.3	»
7)	Manganese chloride Mnc12, 4H20	0.008	»
8)	Cobaltus chloride CoC12, 6H20	0.00138	»
9)	Glucose	10	»
10)	Glycerol	100	»
11)	Distilled water	100	ml.

pH=6.8

The amount of tuberculin produced emploing the above medium was not satisfactory because after its purification and ten times concentration the maxi-

mum tuberculoprotein yield was only 1.5 to 3 mg/ml. We carried out experiments in which some modifications were made in commonly used medium by adding some chemicals to obtain higher rates of the protein content. In this paper we report the results of the experiments carried out in a large scale tuberculin production.

Materials and methods:

Strain: Mycobacterium tuberculosis var. bovis strain AN5 obtained from Mr. D.B. Lee (Weybridge, England)
Medium: Modified Dorset-Henley
Conical culture flasks (Fernbach) for large scale production

Animal: Twenty sensitised albinos male guinea pigs, weighing each 500 to 600.g

In order to obtain pellicle, the organisms were successively passaged in the modified Lowenstein-Jensen (3) and Stonebrink (10) media. Subculture was made on potato medium containing Watson-Reid liquid medium (11). The pellicles were transferred to the liquid synthetic Dorset-Henley medium in erlinmayers as the seed culture and kept for one month at 37°C. To seven series of conical culture flasks each containing one litre of D.H. medium, different chemicals such as calcium pantothenate, sodium pyruvate and sodium glutamate were added. These salts has been used for enrichement of-the media for culturing M. leprae by Murohachi (9). With regard to the similarities between these two organisms, we decided to examine the effect of these salts in the production of Bovine tuberculin. Subculturing was carried out by transferring the pellicles from the seed culture on the surface of the medium in each serie and kept for a period of 2.5 months at 37°C. At the end of this time the microorganisms were heat killed and infused at 100°C for 3 hours. After separating the bacteria by Buchner funnel and sterilising by Seits filter, tuberculoproteins were precipitated by trichloracetic acid at 4°C (8). Protein concentration was determined by Kjeldahl method (4). Biological titration and standardisation was carried out on guinea pigs previously sensitised with bovine tuberculosis str. AN5(6)

Results:

The amount of tuberculin produced in different series after purification and concentration is shown in the following table.

In the seventh serie where sodium glutamate had been added, the growth was very weak and after 4 weeks the microbial veil sank, therefore,-no more experiments was carried out. It seemed that sodium glutamate had inhibited the growth of the organisms. Tuberculoprotein produced after biological titration had a potency of 118% of the English standard of Bovine tuberculin, which has a protein concentration of 1 mg/ml.

Serie NO.	Date of culture	Date of filtration	Materials added		Tuberculin in mg/ml.
			chemicals	mg/ml.	
1	21.1.75	6.7.75	—	—	3.2
2	6.7.75	20.9.75	Ca pantoth.	0.2	8
3	29.7.75	4.10.75	Ca pantoth.	0.1	8.4
4	29.7.75	11.10.75	Na pyruvate	0.1	8.6
5	29.7.75	11.10.75	Mixture of	0.05	
			Ca pan. + Na py	0.05	8.1
6	29.7.75	19.10.75	Ca pan + Na py.	0.1 + 0.1	7.9

Discussion:

The production of Bovine tuberculin takes a longe time, at least 6 months. Therefore if one can impose some changes on the growth medium for more tuberculin production, a lot of time and material will be saved. In search for an answer to this question we have investigated the result of the addition of calcium pantothenate, sodium-pyruvate and sodium glutamate to the synthetic medium. In our experiments it was observed that addition of 0.1 mg/ml. of sodium glutamate hinders the growth of the M. tuberculosis var. bovis and destroys the tuberculosis pellicle. Addition of 0.1 mg/ml. of calcium pantothenate and or sodium pyruvate increases twice the amount of tuberculin as in the conventional medium. When both calcium pantothenate and sodium pyruvate were added into the medium some increase in tuberculoprotein was obtained, provided that the addition does not exceed a certain amount. But addition of each salt alone had a better effect. It has been observed that addition of these chemicals in the amounts of more than one mg/ml. of culture media has decreased the production of tuberculoproteins. Another effect of the addition of these salts is to keep in better condition, the pellicles on the surface of the liquid medium. In one experiment although the bacterial growth was complete after two months, by adding these salts, pellicles remained on the surface of the liquid medium more than four months, in media without additives, pellicles usually sinks after 2.5 months and the growth stops. In another experiment addition of sodium glutamate, calcium pantothenate and sodium pyruvate altogether resulted in a lower rate of cultural growth and a smaller amount of tuberculin production as compared with the usual media.

Acknowledgement:

We wish to thank Mr. D.B. Lee head office of tuberculin section of biological products, standard departements of Cent. Vet. Lab. Weybridge England, for generously providing the strain of bovine tuberculosis and standard bovine tuberculin; and also to Mr. Nikbin, A, Mr. Khadivi, P. and Mr. Mirhachemi, B. for their tecnical assistace.

REFERENCES

1. Dorset (1934).
Comparison of Koch's Old Tuberculin with a New Synthtetic Medium.
J. Am. Vet. M.A. 84, 439.

2. Dorset, M, and Henley, R.R. (1934).
A Synthetic Medium for B. Tuberculosis with a Description of the Method of Producing Tuberculin.
Biochemic Division, U.S. Bureau of Animal Industry, (Nov. 7, 1934).

3. Hedayati, H. Sadri, M., and Zali, M.
Modification of Lowenstein-Jenson Medium for Isolation and Propagation of Bovine Tuberculosis.

In press

4. Jacobs M.B. (1951).
Micro-Kjeldahl Method for Biologicals
Journal of the Am. Ph. Asso. Vol., XL, No. 3 March,

5. Lee, D.B. (1974).
The preparation of Weybridge Bovine Tuberculin
Cent. Vet. Lab. , Weybridge England.

6. Lesslie, I.W. (1960).
Method of Assay of Tuberculin in Guinea Pigs.
Cent. Vet. Lab. Weybridge, England.

7. The Preparation of Weybridge P.P.D. Tuberculin.

7. Lesslie, I.W. (1961).
The Preparation of Weybridge Bovine Tuberculin
Personal communication.

8. Magnusson, M., Bentzon, M. (1958).
Preparation of purified Tuberculin R.T.23.
Bull., Wld. Hlth. Org. 19, 828-843.

9. Murohachi, T. and Yoshida, K. (1976).
Attempts to culture M. leprae on Seimi - Synthetic Solid Agar Media
Acta Leprologica Nouvelle Serie No. 65.

10. Stonebrink, B. (1951).
Thesis, Utrecht, Van Gorcum and comp. N.V.

11. Watson, E.A. (1935).
Watson-Reid Medium
Canad. Pub. Hlth. J, 26, 268.