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## Modification of Fletcher's Medium

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FLETCHER (1928) described a semi-solid medium suitable for the isolation and maintenance of leptospira. He added peptone and meat extract in the medium base and rabbit serum in an amount of 8—10 %.

In the present work trials for modification of this medium was carried out. These were based on the addition of ammonium chloride salt to the base and glucose to the whole medium. JOHNSON and GARY (1962) obtained successful results in substituting rabbit serum with ammonium chloride, and found that *L. pomona* grew well in such medium. ELLINGHAUSEN and McCULLOUGH (1965) reported that ammonium chloride stimulated leptospiral growth. ELLINGHAUSEN (1968) proved that glucose was also stimulatory for lep-

tospirial growth. Therefore, ammonium chloride and glucose were selected for these trials of modification.

### Material and methods

The original FLETCHER's medium was modified by the addition of ammonium chloride salt at rate of 0.5 gm. per litre to the medium base, and glucose at rate of 3.6 gm. per litre. Ammonium chloride was added to the medium base and sterilized with the whole medium, while glucose was sterilized separately by millipore filter and disks of 0.22  $\mu$  porosity before being added to already sterilized medium base. Sterile 10 % rabbit serum was added to both, also various concentrations of rabbit serum (3, 5, 7 or 10 %) were added to the modified medium and compared

**Table 1: Modification of Fletcher's medium with ammonium chloride and glucose**

Serotypes	Number <sup>1</sup> of repetition	Ammonium chloride				Control <sup>3</sup>	
		With glucose		Without glucose		N.P.T.	%
		N.P.T. <sup>2</sup>	%	N.P.T.	%		
<i>L. grippotyphosa</i>	10	38	95	33	82.5	32	80
<i>L. icterohaemorrhagiae</i>	10	36	90	32	80	30	75
<i>L. canicola</i>	10	38	95	35	87.5	34	85

<sup>1</sup> Every repetition entailed inoculation of four tubes.

<sup>2</sup> Number of positive tubes showing the characteristic leptospiral ring.

<sup>3</sup> Ordinary Fletcher's medium with 10 % rabbit serum.

with the original one. Four tubes from each medium were inoculated with 0.5 ml of well grown cultures of *L. grippotyphosa*, *L. icterohaemorrhagiae* and *L. canicola*. All tubes were incubated at 30° C and examined daily, from the third up to the 10th day, macroscopically for the presence of typical ring and microscopically for the presence of leptospira.

Subculturing in modified and original media were repeated for 10 times with 10 days intervals.

### Results

As seen in table 1, the best result was obtained by using FLETCHER'S medium containing both glucose and ammonium chloride. In such medium 95 %, 90 % and 95 % of tubes inoculated with *L. grippotyphosa*, *L. icterohaemorrhagiae* and *L. canicola* respectively, showed growth with the formation of the characteristic leptospiral ring. Tubes containing ammonium chloride, but not glucose showed a corresponding values of 82.5 %, 80 % and 87.5 %, while the original medium provided growth of the three leptospiral species in 80 %, 75 % and 85 % of the tubes.

Data in table 2 reveals that the modified FLETCHER'S medium with ammonium chloride and glucose gave better results in comparison with the original one irrespective of the percentage of rabbit serum added. However, the maximum number of tubes showing the characteristic ring of leptospiral growth was obtained with the modified medium containing 10 % rabbit serum, where 95 %, 90 %, and 95 % positive tubes of *L. grippotyphosa*, *L. icterohaemorrhagiae* and *L. canicola* were shown respectively. The original medium with 10 % rabbit serum demonstrated positive growth in 80 %, 75 % and 85 % of the tubes.

Table 2: Comparison between modified and original Fletcher's medium with different concentrations of rabbit serum

Serotypes	N.R. <sup>1</sup>	Modified Fletcher's medium						Original Fletcher's medium									
		3 % <sup>2</sup>		5 %		7 %		10 %		3 %		5 %		7 %		10 %	
		N.P.T. <sup>3</sup>	%	N.P.T.	%	N.P.T.	%	N.P.T.	%	N.P.T.	%	N.P.T.	%	N.P.T.	%	N.P.T.	%
<i>L. grippotyphosa</i>	10	5	12.5	24	60	33	82.5	38	95	3	7.5	12	30	25	62.5	32	80
<i>L. icterohaemorrhagiae</i>	10	3	7.5	22	55	32	80	36	90	1	2.5	8	20	22	55	30	75
<i>L. canicola</i>	10	5	12.5	23	57.5	37	92.5	38	95	2	5	9	22.5	24	60	34	85

<sup>1</sup> Number of repetition, N.B. every repetition entailed inoculation of four tubes.

<sup>2</sup> Percentage of rabbit serum added to the medium base.

<sup>3</sup> Number of positive tubes, out of 40 tubes, showing the characteristic leptospiral ring.

### Discussion

Since the description of FLETCHER's medium in 1927, several authors tried to formulate media for cultivation of leptospira. All these media contain rabbit serum which was realized to be essential for the growth of leptospira. BABUDIERI and ZARDI (1959) reported that it was not possible to cultivate pathogenic leptospira in a durable manner unless rabbit serum was incorporated in the medium. On the other hand, VOGEL and HUTNER (1961) proposed a chemically defined medium and claimed that it was suitable for growth and maintenance of leptospira. JOHNSON and GARY (1962) stated that the serum could be replaced with ammonium ions. ELLINGHAUSEN and McCULLOUGH (1965) proposed a new serum free medium and reported that it supported the growth of 14 leptospiral serotypes.

In the present work modification of FLETCHER's medium which is a serum containing one, was undertaken by adding ammonium chloride and glucose. Ammonium chloride alone and in combination with glucose enhanced the growth of leptospira; however, they could not replace the rabbit serum completely. Although the modified medium was always better than the original one, the efficiency of both media was significantly reduced when the rabbit serum was added in lower percentage.

Therefore, these results substantiate those obtained by BABUDIERI and ZARDI (1959), JOHNSON and GARY (1962) and ELLINGHAUSEN and McCULLOUGH (1965) as regard to the stimulatory effect of serum, ammonium chloride and glucose; and therefore the modified medium has the advantage that it contains all these three ingredients.

### Summary

Trials for modification of FLETCHER's medium has been undertaken by adding glucose at rate of 3.6 gm./litre and ammonium chloride salt at rate of 0.5 gm./litre. It was found that *L. grippotyphosa*, *L. canicola* and *L. icterohaemorrhagiae* grew better on the modified medium than on the original FLETCHER's medium, even when rabbit serum was reduced to 7 %.

### References

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