NOTE ON THE PLATE METHOD FOR ENUMERATION OF BACTERIA.

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In recent years much work has been done on the bacterial content of milk, chiefly with a view to demonstrating the principal sources of contamination and at the same time collecting data to aid in the fixing of a bacterial standard for "clean" milk. On looking through the various publications one is particularly impressed by the lack of uniformity in the length of the period of incubation of gelatine and agar plates. Some observers adopt a period of only a few days while others prefer to incubate for 8–10 days before counting their plates. It does not yet seem to be generally recognised, in Britain at all events, that, if the highest figures possible are to be obtained, the incubation period must be longer than three days.

Further in such publications, especially where the longer period has been adopted, one too frequently finds that in a series of comparative tests one or more of the gelatine plates has become entirely liquefied and uncountable before the end of the incubation period has been reached, and in this way the whole series has been more or less spoiled. Such upsetting results could, however, be entirely avoided by the simple method, suggested by Hiltner and Störmer[1], of touching the liquefying colonies while still young with the fine point of a stick of silver nitrate, when, if the medium contains a chloride, a white precipitate of silver chloride forms round each colony and prevents further development. The chief difficulty in the carrying out of this suggestion lies in the recognition of the liquefying colonies while they are still so young that they have not excreted any appreciable amount of the liquefying enzyme. Still, with a little practice this difficulty can be very easily surmounted.

[1] *Arb. a. d. biol. Abt. d. Kais. Gesundh.-Amts.* III. p. 449, 1903.

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The work discussed in this note was undertaken with a view to:

(1) Demonstrating the length of time required for the incubation of gelatine and agar plates in order to secure the maximum development of colonies.

(2) Showing the advantages of treating liquefying colonies on gelatine plates with silver nitrate in order to retard development.

(3) Incidentally the numbers of bacteria developing on agar at 22° C. and at 37° C. and on gelatine plates have been compared.

The materials employed for the enumerations were soil, dung and milk and ordinary 12% gelatine and 1·5% agar, made just alkaline to litmus, were used as media. Dilutions were made in the usual way and the plates showing a convenient number of colonies for counting were selected. As the results are merely intended for demonstration purposes and do not bring out anything new, it was not thought advisable to accumulate a great number of data.

Table I shows the length of time required for the number of colonies on the various plates to reach a maximum[1].

In the case of the gelatine plates it will be seen that the maximum for soil and dung is reached in about five days; for the milk sample, however, about nine days elapsed before the maximum was attained.

TABLE I.

No. of days	Gelatine			Agar at 22° C.			Agar at 37° C.		
	Soil	Dung	Milk	Soil	Dung	Milk	Soil	Dung	Milk
1	—	—	—	—	—	—	—	—	—
2	20	48	14	11	7	24	29	20	171
3	57	104	44	17	13	59	36	23	186
4	72	115	49	34	20	88	36	23	—
5	76	117	50	43	26	95	36	24	—
6	76	117	50	55	32	97	36	24	—
7	76	117	51	59	39	100	Plates	dry	—
8	78	118	55	60	44	100			—
9	78	118	61	61	45	100			—
10	79	118	62	61	50	101			—

On agar at 37° C. the maximum was reached in each case in three days. On the third day the plate made from the milk sample was placed in the 22° C. incubator for seven days. This resulted in an increase from 186 to 222 or about 20%.

[1] The dilution employed varies, so that the figures are not comparable for the bacterial content of the materials. This is dealt with later.

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On agar at 22° C. a maximum was not attained till the 8th to 10th day, and the dung plate required to be incubated two days longer, during which time it increased from 50 to 57. After ten days the milk plate was incubated further at 37° C. but no increase in the number of colonies took place.

It is specially desired to emphasise the importance of 8–10 days' incubation for agar plates at 22° C. as this appears to be a point which is frequently overlooked. Doubtless, when comparative figures only are required, three days at 22° C. may suffice but in all other cases it is absolutely essential to incubate for at least eight days. 10 days' incubation as compared with three has given an increase varying from about one to three times. For gelatine plates 8–10 days' incubation is required and for agar at 37° C., three days.

In Table II a comparison is made between the number of colonies developing on ordinary gelatine plates and the number obtained on gelatine plates on which the liquefying colonies have been treated with silver nitrate while still small.

TABLE II.

	Soil		Dung		Milk	
No. of days	Gelatine untreated	Gelatine with silver nitrate	Gelatine untreated	Gelatine with silver nitrate	Gelatine untreated	Gelatine with silver nitrate
1	—	—	—	—	—	—
2	21	20	46	48	18	14
3	Plate liquefied	57	96	104	52	44
4		72	120	115	54	49
5		76	136	117	59	50
6		76	Plate liquefied	117	61	50
7		76		117	63	51
8		78		118	Plate liquefied	55
9		78		118		61
10		79		118		62

The advantages of this method of treatment are most obvious in the cases of soil and milk, in both of which the ordinary plate was liquefied before the other had attained its maximum. This is particularly marked in the case of soil, which, however, was only to be expected as here one is dealing with a medium containing large numbers of liquefying moulds. In the enumeration of bacteria in soil, therefore, the method of treating liquefying colonies with silver nitrate is specially to be recommended.

It should be observed that in practically every case the plate treated with silver nitrate has shown a slightly smaller number of

colonies than the untreated plate, *i.e.* comparing the numbers on the same day. This may possibly be due to the effect of the necessarily considerable quantity of silver chloride on the treated plate. Still one must observe that on the average a higher number will be found on the treated plate at the end of incubation than on the untreated before liquefaction.

Table III gives the numbers of bacteria per gram or cubic centimetre of the material as indicated by the growth of colonies on gelatine and agar plates.

TABLE III.

	Soil (gram)	Dung (gram)	Milk (c.c.)
Gelatine	7,900,000	1,180,000,000	6,200,000
Agar at 22° C.	6,100,000	570,000,000	1,010,000
Agar at 37° C.	360,000	2,400,000	222,000

It is particularly noteworthy that in each case the highest numbers are obtained from gelatine plates; next comes agar at 22° C. and lastly agar at 37° C. These results are very much as one might expect from a consideration of the sources of the bacteria in each material. In soil in temperate climates the organisms are accustomed to low temperatures and so might be expected to grow well at 22° C. Thus the two plates (gelatine and agar) incubated at 22° C. gave very nearly the same result, while that kept at 37° C. gave a very much lower figure. In the case of dung, if the material is fresh (as was the case in the sample examined), the same remarks apply: but in an old rotting sample the bacteria which thrive best at 22° C. do not flourish and are replaced by the multiplication of others which are better adapted to the high temperature prevailing in the material. In an ordinary sample of market milk one might also expect the greatest development at 22° C., since most of the bacteria in such samples are derived from the air and from the skin of the cow and are thus accustomed to temperatures in the neighbourhood of 22° C. This view is supported by the fact that the 37° C. agar plate on incubation at 22° C. showed a further increase, whereas the numbers on the 22° C. agar plate remained stationary when the latter was incubated at 37° C. (see above).

Further, dung contains considerable quantities of easily decomposable nitrogenous material and, therefore, one may presume, bacteria specially suited to a highly nitrogenous medium. This may partially account for the optimum growth of bacteria on gelatine. In the case of soil, although the content of nitrogenous organic material is not great, we

know from ammonification and other experiments that large numbers of organisms are present which might be expected to flourish on a nitrogenous medium.

Considering that gelatine appears to be such an excellent medium for the growth of bacteria from soil, dung and milk there would seem to be no adequate reason why it should not be more frequently employed especially as the introduction of the silver nitrate method for the killing of liquefying colonies has made it practically as permanent as agar.

In conclusion, I wish to thank Prof. Löhnis for having suggested this piece of work and also for advice and suggestion during the carrying out of the same.