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# THE KEEPING QUALITY OF SMALLPOX VACCINE

by

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This report deals with a series of comparative laboratory trials of the keeping quality of dry and wet smallpox vaccines carried out under the auspices of the World Health Organization. A report on the bacterial purity of the vaccines tested will be found as Annex I.

## Materials and Methods

Each of the four producing laboratories (designated by the letters W, X, Y, Z) was supplied with a sufficient quantity of a single batch of dried vaccine and of a wet preparation (glycerinated lymph) prepared from the same strain of vaccinia. The vaccines were shipped by air on dry ice to the three testing laboratories (designated by the letters A, B, C), each vaccine being tested in two laboratories.

A single batch of control (wet) vaccine was supplied to all testing laboratories. This was held below -10°C throughout, samples being withdrawn at the appropriate time for use in each test.

The samples of the test vaccines, both wet and dry, were stored at 0°C (0°-4°C), 22°C (19°-23°C), 37°C, and 45°C. Potency tests were carried out at the following intervals.

| Material stored at | Tested |
|---|---|
| 0°C | On receipt and at 4, 12, 24 and 52 weeks |
| 22°C | at 2 and 4 weeks, then at 4-week intervals |
| 37°C | at 2, 4, 6 and 8 weeks, then at 4-week intervals |
| 45°C | at 1, 2, 4, 6 and 8 weeks, then at 4-week intervals. |

In the event that a test could not be carried out at the proper time, the sample was removed from the incubator and stored at $0^{\circ}C$ or below until the test could be performed.

Potency testing was carried out according to the following technique. The dilutions used for both test and control vaccines were normally 1 in 100, 1 in 1000, 1 in 3000, 1 in 9000 and 1 in 27 000. When the 1 in 1000 dilution failed to give rise to a confluent or coalescent lesion, the undiluted vaccine and a dilution of 1 in 10 was tested in subsequent tests as well as the 1 in 100 and 1 in 1000 dilutions.

The diluent was an isotonic buffered solution at or near neutrality. Two rabbits were used for each titration, a quantity of 0.1 ml of the appropriate dilution being spread over a clipped area of skin of 5 $cm^2$ (alternatively, 0.2 ml was spread over 10 $cm^2$) and the skin then lightly scarified through the fluid.

The dilutions of all three vaccines (wet, dry, and control) were applied to each rabbit. Thus differences in end-point between rabbits of the same pair could be used for estimating experimental error when comparing vaccines tested in the same laboratory after storage at the same temperature.

The results were recorded as follows:

C = Confluent, lesions covering the whole area

SC+ = Semi-confluent+, lesions covering 70-80% of the area

SC = Semi-confluent, lesions coalescing but covering 50-70% of the area

SC- = Semi-confluent-, lesions coalescing but covering substantially less than 50% of the area

Countable, the actual number of vesicles is stated.

Tests were continued until a vaccine failed to produce consistent confluent reactions at 1 in 100 dilution. Precise details of the duration of the tests are given in Tables 2, 4 and 5.

Statistical Analysis of Scarification Tests

In the tables and calculations which follow the negative logarithms to the base 10 of the dilutions are used, viz.

1/100-2, 1/1000-3, 1/3000-3.477, 1/9000-3.954, 1/27 000-4.431

These are here called "titres".

Two end-points were used for statistical analysis:

(1) A point midway between the titres corresponding to C and SC+. More precisely this is the mean of the highest titre which gave C and the lowest which gave SC+. If either of these latter figures was not available, an estimate was made by extrapolation.

(2) A point midway between SC- and "countable". This was obtained similarly.

Average Rates of Decline in Potency

The numerical values representing the end-points chosen are positive and increase with the potency of the vaccine. For each vaccine at each storage temperature the linear regressions coefficients of the end-points (means of two rabbits) on time of testing were calculated over the whole period of testing. These are in general negative, and give the average rates of decline per week over the whole period. The rate of decline was not of course constant over the whole period, and the wet vaccines lost their potency much more quickly than the dry. In order to make unbiased comparisons possible, the average rates of decline for the dry vaccines were also calculated for the same period as for the wet vaccines.

The end-points as defined are the logarithms of dilutions which correspond to a specific response. The quantities D - S, W - S, where D, W, S are the respective values of the first end-point (or of the second) for the dry, wet and standard (control) vaccines, are measures of the potencies of D and W relative to the standard. (In fact, they are the logarithms of estimates of the relative potencies.) The rates of decline of D - S and W - S were calculated in the same way as those of D and W.

All these results with their standard errors are shown in Tables 4, 5, 6, 7. Curves of Response, showing the variation of the end-points with time (Figs. 1, 2, 4), or of the potency ratios based on them (Fig. 3), are also given. In Figures 1, 2, 4 showing end-points, the vertical distance between two curves from the same laboratory has to be more than about 1.1 to be statistically significant at the 5% level. In Fig. 3, showing log-potency-ratios the corresponding difference is 1.5. For curves from different laboratories the corresponding figures are 1.3, 1.8.

Behaviour of the Control Vaccine

The control vaccine showed no significant deterioration at any of the temperatures tested.[1]

It follows therefore that the rates of decline for the dry (D) and wet (W) vaccines give the same information as those for the relative potencies, D - S and W - S.

It is also of some importance to examine whether or not the absolute level of response to the control is effectively the same for all tests in one laboratory, or in different laboratories. In the tests carried out at laboratory B the responses to the control vaccine at all times were reported as confluent. Hence no end-points could be calculated. Therefore, it is only for the tests carried out at laboratories A and C (Lister) that the controls can be compared in this way.

Table 1 shows the comparison for the vaccine prepared in laboratory Y. The overall mean values (averaged over the entire test) for the end-points are tabulated,

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[1] The standard errors tabulated in Tables 4, 5, 6, 7, are those appropriate for comparisons between the three vaccines, they do not allow for variation between rabbits. In spite of this, it is interesting to examine the rates of deterioration for the control vaccine which are "significantly different" from zero. There are 4 such values for the first end-point and 9 for the second. Of the former, 3 are negative and 1 positive; of the latter, 8 are negative and 1 positive (the negative sign signifies deterioration). Such differences may safely be attributed to rabbit variation.

together with their standard errors. Table 2 gives the other possible comparisons. There are no significant differences in the average level of response to the control vaccine in either testing laboratory but the values from laboratory A are a little lower (about 0.5) than the values from laboratory C.

## Comparative Effects of Temperature

None of the dry vaccines showed appreciable deterioration at 0-4°C. The three wet vaccines tested at laboratory B showed some deterioration at this temperature, but this was confirmed by the other testing laboratory only for the vaccine from laboratory Z. The wet vaccine deteriorated far more quickly than the dry.

Table 3 shows the time in weeks from the beginning of the trial to the last tests for which an end-point could be obtained.

TABLE 3

Time in weeks from the beginning to the last test for which an end-point could be obtained

| Vaccine tested at | | Vaccine prepared at | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | W | | X | | Y | | Z | |
| | | D | W | D | W | D | W | D | W |
| A | 0-4°C | 52 | 52 | | | 52 | 52 | | |
| | 22°C | 20 | 24 | | | 45 | 28 | | |
| | 37°C | 4 | 0 | | | 20 | 4 | | |
| | 45°C | 4 | 0 | | | 6 | 4 | | |
| B | 0-4°C | 104 | 24 | 104 | 52 | | | 104 | 52 |
| | 22°C | 32 | 4 | 104 | 4 | | | 44 | 4 |
| | 37°C | 4 | 1 | 12 | 1 | | | 8 | 1 |
| | 45°C | 6 | 0 | 6 | 0 | | | - | - |
| C | 0-4°C | | | 52 | 52 | 52 | 52 | 52 | 52 |
| | 22°C | | | 68 | 12 | 68 | 20 | 68 | 16 |
| | 37°C | | | 87 | - | 16 | 4 | 16 | 0 |
| | 45°C | | | 24 | - | 12 | 2 | 6 | 0 |

Vaccine prepared at W

22°C. The wet vaccine tested at A showed a slow but steady deterioration over 24 weeks, whereas the dry showed no significant deterioration at 20 weeks using C to SC+ and relatively little using "SC- to Countable"; when the same vaccines were tested at B, the wet vaccine showed marked deterioration after 4 weeks, and the dry steady deterioration over 32 weeks.

At 37° and 45° the wet vaccine tested in both places went off almost at once, the dry showed complete deterioration at the end of 4 weeks.

Vaccine prepared at X

22°C. The wet vaccine tested at C had deteriorated sufficiently for the test to be terminated after 12 weeks. During this period the rate of deterioration was steady. When the same vaccine was tested at B, the test came to an effective end after 4 weeks, over this period the rate of deterioration was similar in both laboratories.

The dry vaccine tested at C showed no significant deterioration after 68 weeks, the dry vaccine tested at B showed a slow deterioration over 104 weeks.

37° and 45°. At 37° and 45° the wet vaccines in both laboratories went off almost at once.

Vaccine prepared at Y

22°C. The wet vaccine tested at A showed a slow but steady deterioration over 28 weeks; the corresponding vaccine tested at C deteriorated at about the same weekly rate over 20 weeks. The dry vaccine tested at A showed a little deterioration, but considerably less than the wet even over the longer period of 45 weeks; the corresponding vaccine tested at C showed similar deterioration. The rates of deterioration were not significantly different in the two laboratories.

37° and 45°. In both laboratories the wet vaccine had gone off completely in 4 weeks or less; while the dry vaccine also showed marked deterioration in the same period, the latter vaccine deteriorated further in the course of another 16 weeks, but about half the deterioration at 37° and more at 45° had taken place in the first month.

Vaccine prepared at Z

22°C. The wet vaccine tested at C had gone off by the end of 16 weeks; the test of the corresponding vaccine at B came to an end after 4 weeks during which the rate of deterioration had been relatively rapid. The dry vaccine tested at C did not deteriorate greatly even over 68 weeks; the dry vaccine tested at B deteriorated at a rather faster rate over 44 weeks.

37° and 45°. No information on rate of deterioration is available at these temperatures for the wet vaccine tested at C, nor for that tested at B at 45°. Curiously enough, on the basis of the second end-point (SC- to Countable), the wet vaccine tested at B at 37° showed no significant deterioration after one week (after which no end-points could be calculated) while the dry vaccine, on the other hand, did show deterioration; even on the basis of the first end-point the wet deteriorated as much as the dry. The dry vaccine showed further but not complete deterioration after 8 weeks at 37°C.

The dry vaccine tested at C showed great deterioration after 16 weeks at 37°C. The deterioration after 6 weeks at 45°C was still greater.

Comparison of Laboratories

In Figs. 3(a), (b), (c), (d), the quantities D-S and W-S have been plotted against time of testing for the vaccines prepared at Y and tested at A and C. The results show no significant differences in the absolute levels or in the rates of deterioration, relative to the standard of corresponding vaccines. (See also Table 1 for absolute levels.)

Figs. 1, 2, 4, refer to vaccines prepared at W, X and Z. Since no end-points could be calculated from the observations at B on the control vaccine, the quantities D, W have been plotted for both testing laboratories and S for the laboratory for which it is available. These curves are valid for comparing rates of deterioration in the two laboratories.

We can perhaps assume that the absolute level of response to the control vaccine at B did not differ more from the absolute levels of response to the standard in the

other two testing laboratories than they differed among themselves. If so, it can be inferred that the levels of potency of corresponding vaccines tested at B and A, or at B and C are not significantly different. This is certainly true of corresponding rates of deterioration.

Comparison between Vaccines

The comparison between vaccines prepared in different centres should be made principally on the dry preparations; the wet preparations certainly deteriorated at a much faster rate than the dry and have therefore less practical importance. A summarized comparison of the dry vaccines follows:

Temperature 22°C

Vaccine W

The dry vaccine tested at A showed no significant deterioration at 20 weeks using "C to SC+" as an end-point and relatively little using "SC- to Countable", the dry vaccine tested at B showed steady deterioration over 32 weeks.

Vaccine X

The dry vaccine tested at C showed no significant deterioration after 68 weeks; that tested at B showed a slow deterioration over 104 weeks.

Vaccine Y

The dry vaccine tested at A showed a little deterioration over 45 weeks; that tested at C showed similar deterioration.

Vaccine Z

The dry vaccine tested at C did not deteriorate greatly even over 68 weeks. The dry vaccine tested at B deteriorated at a rather faster rate over 44 weeks.

Temperature 37° and 45°C

Vaccine W

The dry vaccine showed complete deterioration at the end of 4 weeks.

Vaccine X

The dry vaccine tested at C did not deteriorate completely until after 87 weeks at 37°C and after 24 weeks at 45°C. At B the tests on the dry vaccine were discontinued after 12 weeks at 37°C and after 6 weeks at 45°C; by this time they had apparently reached just as low levels as at the termination of the tests at C.

Vaccine Y

The dry vaccine showed marked deterioration over 4 weeks, and deteriorated further in the course of another 16 weeks; half the deterioration at 37° and more at 45° had taken place in the first month.

Vaccine Z

The dry vaccine tested at C showed great deterioration after 16 weeks at 37°C. The deterioration after 6 weeks at 45°C was still greater.

The dry vaccine tested at B deteriorated at about the same rate as the former vaccine.

There is no doubt that the vaccine prepared at X was the most stable and that prepared at W the least stable. The vaccines prepared at Y and Z are intermediate in this respect. Between these two preparations there is not a great deal of difference; the results at 22°C suggest a little advantage to the vaccine Y.

The results for the wet vaccines, besides being less important, are also less consistent than those for the dry.

TABLE 1

Standard used in testing vaccines prepared at Y

Overall mean end-points, with standard errors

| Testing Laboratory | C to Sc+ | | | | Sc- to Countable | | | |
|---|---|---|---|---|---|---|---|---|
| | A | | C | | A | | C | |
| 0 - 4 C. | 2.86 (52, | ±0.22 6)* | 3.42 (52, | ±0.09 5) | 3.74 (52, | ±0.14 6) | 4.32 (52, | ±0.17 5) |
| 22 C. | 2.79 (45, | ±0.13 12) | 3.28 (68, | ±0.11 15) | 3.59 (45, | ±0.24 12) | 4.41 (68, | ±0.12 15) |
| 37 C. | 2.95 (20, | ±0.14 8) | 3.59 (16, | ±0.22 7) | 3.98 (20, | ±0.12 8) | 4.63 (16, | ±0.19 7) |
| 45 C. | 3.48 (6, | ±0.31 5) | 3.60 (12, | ±0.22 7) | 4.63 (6, | ±0.47 5) | 4.62 (12, | ±0.18 7) |

* The first number in brackets gives the duration of the test in weeks, the second the number of separate occasions of testing on which the mean is based.

TABLE 2

Standards used in testing vaccines

Overall mean end-points, with standard errors

| | C to Sc+ | | | Sc- to Countable | | |
|---|---|---|---|---|---|---|
| Vaccine from | W | X | Z | W | X | Z |
| Vaccine tested by | A | C | C | A | C | C |
| 0 - 4 C. | 2.96 ± 0.21 (52, 6)* | 3.36 ± 0.10 (52, 5) | 3.40 ± 0.06 (52, 5) | 3.80 ± 0.12 (52, 6) | 4.35 ± 0.10 (52, 5) | 4.33 ± 0.11 (52, 5) |
| 22 C. | 2.65 ± 0.10 (24, 8) | 3.27 ± 0.09 (68, 17) | 3.28 ± 0.12 (68, 16) | 3.59 ± 0.10 (24, 8) | 4.32 ± 0.12 (68, 16) | 4.33 ± 0.11 (68, 16) |
| 37 C. | 2.88 - (4, 2) | 3.30 ± 0.09 (87, 18) | 3.53 ± 0.20 (16, 7) | 3.84 - (4, 2) | 4.36 ± 0.11 (68, 16) | 4.58 ± 0.20 (16, 7) |
| 45 C. | 3.64 - (4, 2) | 3.28 ± 0.10 (24, 10) | 3.76 ± 0.14 (6, 5) | 4.80 - (4, 2) | 4.15 ± 0.09 (24, 9) | 4.79 ± 0.19 (6, 5) |

* The first number in brackets gives the duration of the test in weeks, the second the number of separate occasions of testing on which the mean is based.

TABLE 4(a)

Vaccines prepared at W

Average rates of deterioration per week of dry, wet and standard vaccines (End-point C to Sc+)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by A | | | | | | | | | | | | |
| 0-4°C | ( -.026 | .009 | | | (-.001)* | | -.025 | .013 | | | 52 | |
| | ( | | +.00001 | .008 | +.006 | .008 | | | -.006 | .012 | | 52 |
| 22°C | ( -.009 | .022 | | | -.027 | .022 | +.018 | .032 | | | 20 | |
| | ( | | -.088 | .018 | -.016 | .018 | | | -.072 | .025 | | 24 |
| 37°C | -.470 | .123 | - | - | -.110 | .123 | -.360 | .174 | - | - | 4 | 0 |
| 45°C | -.518 | .052 | - | - | +.272 | .052 | -.790 | .073 | - | - | 4 | 0 |
| Tested by B | | | | | | | | | | | | |
| 0-4°C | ( -.013 | .026 | -.065 | .020 | | | | | | | 24 | 24 |
| | ( -.020 | .004 | | | | | | | | | 104 | |
| 22°C | ( -.105 | .051 | -.352 | .025 | | | | | | | 4 | 4 |
| | ( -.136 | .003 | | | | | | | | | 32 | |
| 37°C | ( | | -1.350 | ** | | | | | | | | 1 |
| | ( -.333 | ** | | | | | | | | | 4 | |
| 45°C | -.693 | .042 | - | - | | | | | | | 6 | 0 |

* One observation for the dry vaccine was missing. This is the result for the standard excluding the value corresponding to the missing one.

** S.E. unobtainable, all pairs of rabbits agreed.

TABLE 4(b)

Vaccines prepared at W

Average rates of deterioration per week of dry, wet and standard vaccines (End-point Sc- to Countable)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by A | | | | | | | | | | | | |
| 0-4°C | (-.017 | .008 | | | (-.004)* | | -.013 | .012 | | | 52 | |
| | ( | | -.001 | .008 | -.001 | .008 | | | -.0002 | .011 | | 52 |
| 22°C | (-.038 | .018 | | | | | -.014 | .025 | | | 20 | |
| | ( | | -.088 | .014 | -.015 | .014 | | | -.073 | .020 | | 24 |
| 37°C | -.388 | .095 | - | - | -.060 | .095 | -.328 | .135 | - | - | 4 | 0 |
| 45°C | -.505 | .106 | - | - | +.418 | .106 | -.923 | .150 | - | - | 6 | 0 |
| Tested by B | | | | | | | | | | | | |
| 0-4°C | ( 0 | .022 | -.081 | .017 | | | | | | | 24 | 24 |
| | (-.015 | .004 | | | | | | | | | 104 | |
| 22°C | (-.015 | .111 | -.418 | .056 | | | | | | | 4 | 4 |
| | (-.108 | .006 | | | | | | | | | 32 | |
| 37°C | ( - | | -.960 | ** | | | | | | | - | 1 |
| | (-.286 | ** | | | | | | | | | 4 | |
| 45°C | -569 | -047 | - | - | | | | | | | 6 | 0 |

* One observation for the dry vaccine was missing. This is the result for the standard excluding the value corresponding to the missing one.

** S.E. unobtainable, all pairs of rabbits agreed.

TABLE 5(a)

Vaccines prepared at X

Average rates of deterioration per week of dry, wet and standard vaccines (End-point C to Sc+)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by C | | | | | | | | | | | | |
| 0-4°C | +.004 | .010 | -.014 | .010 | -.006 | .010 | +.010 | .014 | -.009 | .014 | 52 | 52 |
| 22°C | (+.003 | .046 | -.267 | .046 | -.022 | .046 | +.025 | .644 | -.246 | .644 | 12 | 12 |
| | (-.002 | .005 | | | +.006 | .005 | -.008 | .007 | | | 68 | |
| 37°C | -.035 | .005 | - | - | +.010 | .005 | -.045 | .007 | - | - | 87 | - |
| 45°C | -.154 | .018 | - | - | -.043 | .018 | -.112 | .025 | - | - | 24 | |
| Tested by B | | | | | | | | | | | | |
| 0-4°C | (-.011 | .009 | -.071 | .012 | | | | | | | 52 | 52 |
| | (-.004 | .004 | | | | | | | | | 104 | |
| 22°C | (-.180 | .183 | -.428 | .183 | | | | | | | 4 | 4 |
| | (-.030 | .005 | | | | | | | | | 104 | |
| 37°C | (-.920 | .467 | -1.590 | .467 | | | | | | | 1 | 1 |
| | (-.296 | .039 | | | | | | | | | 12 | |
| 45°C | -.500 | .029 | - | - | | | | | | | 6 | 0 |

TABLE 5(b)

Vaccines prepared at X

Average rates of deterioration per week of dry, wet and standard vaccines (End-point Sc- to Countable)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by C | | | | | | | | | | | | |
| 0-4°C | +.009 | .009 | -.001 | .009 | +.015 | .009 | -.006 | .013 | -.016 | .013 | 52 | 52 |
| 22°C | (-.007 | .034 | -.150 | .034 | +.015 | .034 | -.022 | .049 | -.165 | .049 | 12 | 12 |
| | (-.003 | .003 | | | +.010 | .003 | -.013 | .005 | | | 68 | |
| 37°C | -.028 | .004 | - | - | +.007 | .004 | -.034 | .006 | - | - | 68 | - |
| 45°C | -.110 | .012 | - | - | -.027 | .012 | -.083 | .018 | - | - | 24 | - |
| Tested by B | | | | | | | | | | | | |
| 0-4°C | (-.012 | .007 | -.060 | .009 | | | | | | | 52 | 52 |
| | (-.007 | .003 | | | | | | | | | 104 | |
| 22°C | (-.240 | .117 | -.478 | .117 | | | | | | | 4 | 4 |
| | (-.022 | .003 | | | | | | | | | 104 | |
| 37°C | (-.950 | .313 | -1.19 | .313 | | | | | | | 1 | 1 |
| | (-.303 | .026 | | | | | | | | | 12 | |
| 45°C | -.582 | .052 | - | - | | | | | | | 6 | 0 |

TABLE 6(a)

Vaccines prepared at Y

Average rates of deterioration per week of dry, wet and standard vaccines. (End-point C to Sc+)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by A | | | | | | | | | | | | |
| 0-4°C | -.008 | .009 | -.005 | .009 | -.011 | .009 | -.001 | .012 | +.005 | .012 | 52 | 52 |
| 22°C | (-.054 | .014 | -.081 | .145 | -.022 | .014 | -.031 | .020 | -.058 | .020 | 28 | 28 |
| | (-.027 | .008 | | | -.011 | .008 | -.016 | .011 | | | 45 | |
| 37°C | (-.472 | .148 | -1.000 | .148 | -.132 | .148 | -.340 | .209 | -.868 | .209 | 4 | 4 |
| | (-.176 | .023 | | | -.036 | .023 | -.142 | .032 | | | 20 | |
| 45°C | (-.812 | .188 | -.799 | .188 | +.068 | .188 | -.880 | .265 | -.867 | .265 | 4 | 4 |
| | (-.557 | .115 | | | -.110 | .115 | -.446 | .163 | | | 6 | |
| Tested by C | | | | | | | | | | | | |
| 0-4°C | -.012 | .004 | -.014 | .004 | -.007 | .004 | -.005 | .005 | -.006 | .005 | 52 | 52 |
| 22°C | (-.040 | .022 | -.129 | .022 | +.019 | .022 | -.059 | .031 | -.148 | .031 | 20 | 20 |
| | (-.028 | .005 | | | -.018 | .005 | -.010 | .008 | | | 68 | |
| 37°C | (-.390 | .184 | -1.309 | .184 | +.035 | .184 | -.425 | .260 | -1.345 | .260 | 4 | 4 |
| | (-.224 | .038 | | | +.021 | .038 | -.246 | .053 | | | 16 | |
| 45°C | (-.950 | .260 | -2.620 | .260 | -.090 | .260 | -.860 | .367 | -2.530 | .367 | 2 | 2 |
| | (-.468 | .035 | | | -.070 | .035 | -.398 | .050 | | | 12 | |

TABLE 6(b)

Vaccines prepared at Y

Average rates of detericration per week of dry, wet and standard vaccines. (End-point Sc- to Countable)

| | D | | W | | S | | D - S | | D - W | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by A | | | | | | | | | | | | |
| 0-4°C | -.015 | .006 | -.017 | .006 | -.012 | .006 | -.003 | .009 | -.004 | .009 | 52 | 52 |
| 22°C | (-.056 | .016 | -.062 | .016 | -.018 | .016 | -.038 | .023 | -.044 | .023 | 28 | 28 |
| | (-.031 | .008 | | | -.009 | .008 | -.022 | .012 | | | 45 | |
| 37°C | (-.475 | .108 | -1.03 | .108 | -.018 | .108 | -.458 | .153 | -1.01 | .153 | 4 | 4 |
| | (-.164 | .016 | | | -.035 | .016 | -.129 | .023 | | | 20 | |
| 45°C | (-.646 | .181 | -.823 | .181 | +.276 | .181 | -.922 | .256 | -1.097 | .256 | 4 | 4 |
| | (-.354 | .111 | | | -.052 | .111 | -.301 | .157 | | | 6 | |
| Tested by C | | | | | | | | | | | | |
| 0-4°C | -.008 | .005 | -.011 | .005 | -.020 | .005 | +.012 | .007 | +.009 | .007 | 52 | 52 |
| 22°C | (-.028 | .018 | -.079 | .018 | -.014 | .018 | -.013 | .025 | -.064 | .025 | 20 | 20 |
| | (-.022 | .004 | | | -.019 | .004 | -.003 | .006 | | | 68 | |
| 37°C | (-.288 | .189 | -1.258 | .189 | -.202 | .189 | -.085 | .268 | -1.055 | .268 | 4 | 4 |
| | (-.218 | .039 | | | +.018 | .039 | -.236 | .055 | | | 16 | |
| 45°C | (-.680 | .270 | -2.515 | .270 | -.330 | .270 | -.350 | .383 | -2.185 | .383 | 2 | 2 |
| | (-.451 | .037 | | | -.076 | .037 | -.375 | .052 | | | 12 | |

TABLE 7(a)

Vaccines prepared at Z

Average rates of deterioration per week of dry, wet and standard vaccines (End-point C to Sc+)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by C | | | | | | | | | | | | |
| 0-4°C | -.029 | .007 | -.033 | .007 | -.005 | .007 | -.024 | .010 | -.028 | .010 | 52 | 52 |
| 22°C | (-.070 | .032 | -.280 | .032 | +.015 | .032 | -.085 | .046 | -.295 | .046 | 16 | 16 |
| | (-.031 | .005 | | | -.015 | .005 | -.016 | .008 | | | 68 | |
| 37°C | -.203 | .022 | - | - | +.003 | .022 | -.206 | .032 | - | - | 16 | 0 |
| 45°C | -.649 | .091 | - | - | -.025 | .091 | -.624 | .129 | - | - | 6 | 0 |
| Tested by B | | | | | | | | | | | | |
| 0-4°C | (-.031 | .005 | -.045 | .005 | | | | | | | 52 | 52 |
| | (-.004 | .002 | | | | | | | | | 104 | |
| 22°C | (-.128 | .108 | -.355 | .107 | | | | | | | 4 | 4 |
| | (-.058 | .004 | | | | | | | | | 44 | |
| 37°C | (-.950 | .039 | -.870 | .039 | | | | | | | 1 | 1 |
| | (-.599 | .005 | | | | | | | | | 8 | |
| 45°C | - | | - | | | | | | | | - | - |

TABLE 7(b)

Vaccines prepared at Z

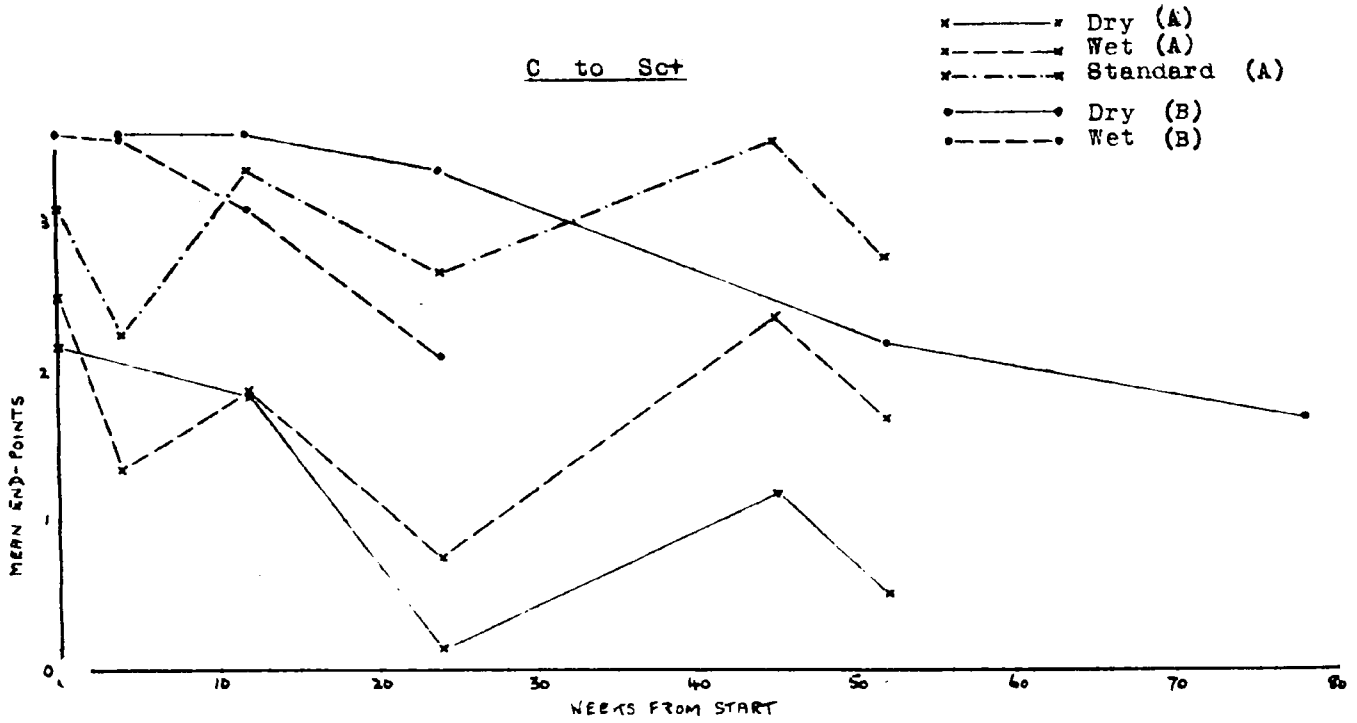
Average rates of deterioration per week of dry, wet and standard vaccines. (End-point Sc- to Countable)

| | D | | W | | S | | D - S | | W - S | | Duration (Weeks) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | Rate | S.E. | D | W |
| Tested by C | | | | | | | | | | | | |
| 0-4°C | -.017 | .008 | -.036 | .008 | -.017 | .008 | +.0003 | .011 | -.019 | .011 | 52 | 52 |
| 22°C | (-.047 | .024 | -.232 | .024 | +.016 | .024 | -.064 | .034 | -.248 | .034 | 16 | 16 |
| | (-.025 | .004 | | | -.014 | .004 | -.012 | .006 | | | 68 | |
| 37°C | -.199 | .025 | - | - | +.011 | .025 | -.209 | .036 | - | - | 16 | 0 |
| 45°C | -.576 | .067 | - | - | -.167 | .067 | -.409 | .095 | - | - | 6 | 0 |
| Tested by B | | | | | | | | | | | | |
| 0-4°C | (-.016 | .006 | -.060 | .005 | | | | | | | 52 | 52 |
| | (-.007 | .002 | | | | | | | | | 104 | |
| 22°C | (-.131 | .174 | -.306 | .172 | | | | | | | 4 | 4 |
| | (-.015 | .006 | | | | | | | | | 44 | |
| 37°C | (-.960 | .229 | -.240 | .229 | | | | | | | 1 | 1 |
| | (-.381 | .029 | | | | | | | | | 8 | |
| 45°C | - | | - | | | | | | | | - | - |

Vaccine prepared at Bandung

Mean End-points for Dry, Wet and Standard Vaccines - 0 -.4°C.

C to Sc+




Sc- to Countable

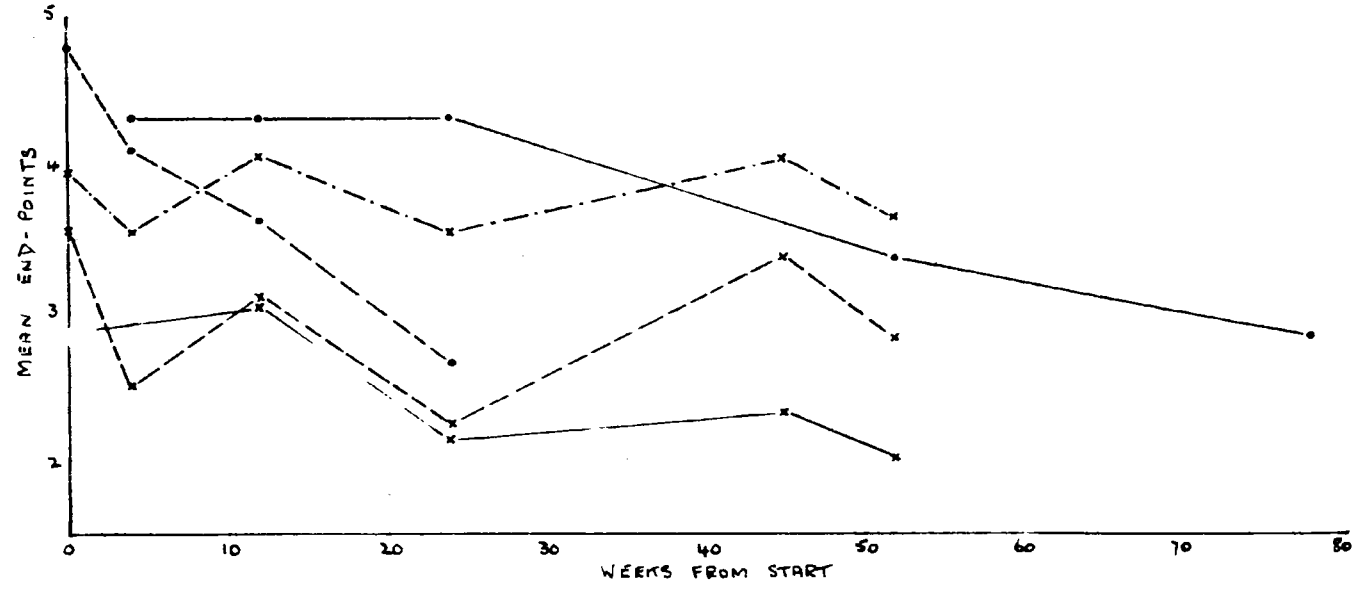



FIG. 1a.

Vaccine prepared at W

Mean End-points for Dry, Wet and Standard Vaccines - 22°C.

Dry (A)
Wet (A)
Standard (A)
Dry (B)
Wet (B)

C to Sc+

Sc- to Countable

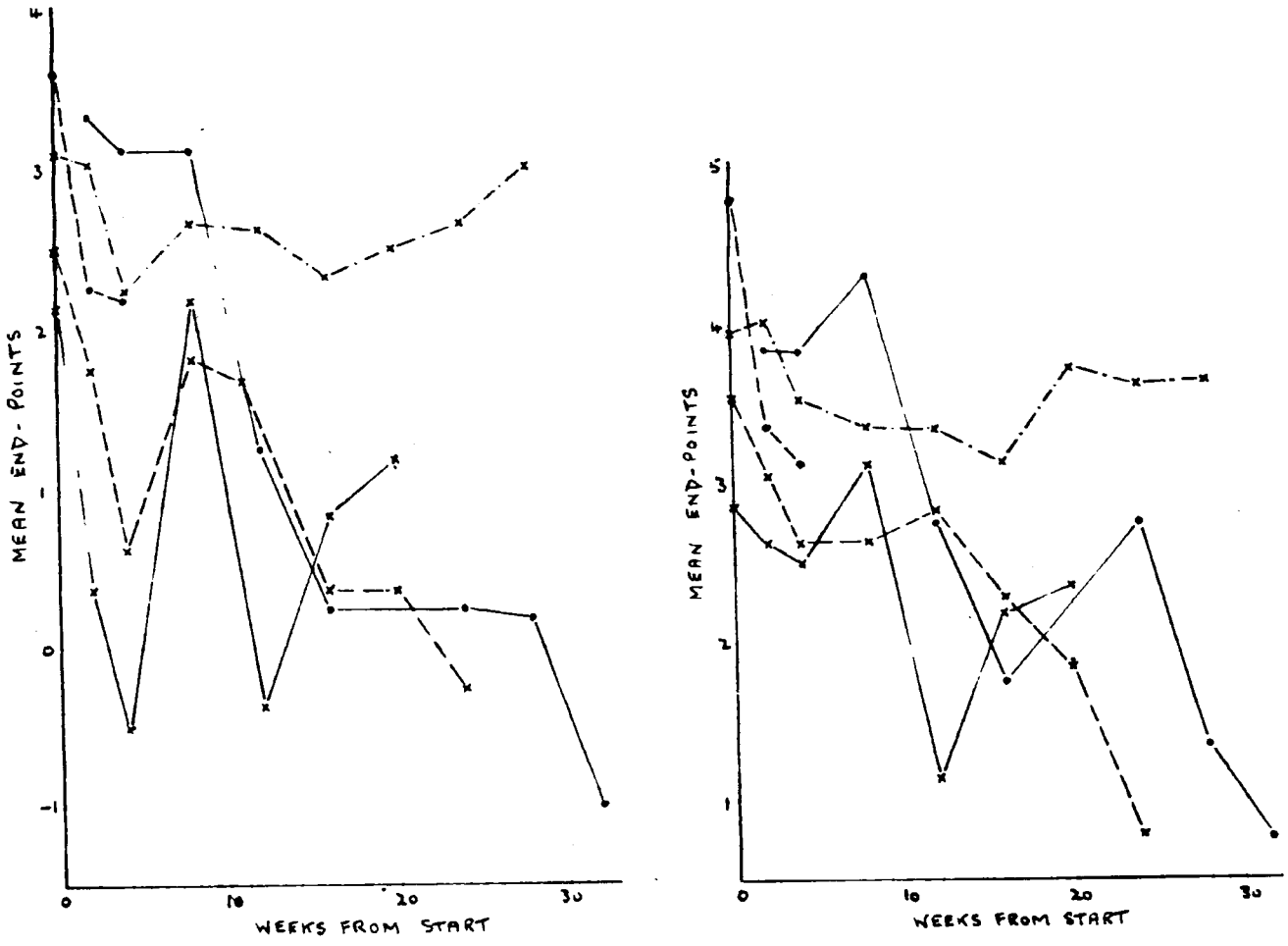



FIG. 1.B.

Vaccine prepared at W

Mean End-points for Dry, Wet and Standard Vaccines - 37° and 45°C.

Dry (A)
Wet (A)
Standard (A)
Dry (B)
Wet (B)

37°C.

C to Sc+

Sc- to Countable

45°C.

C to Sc+

Sc- to Countable

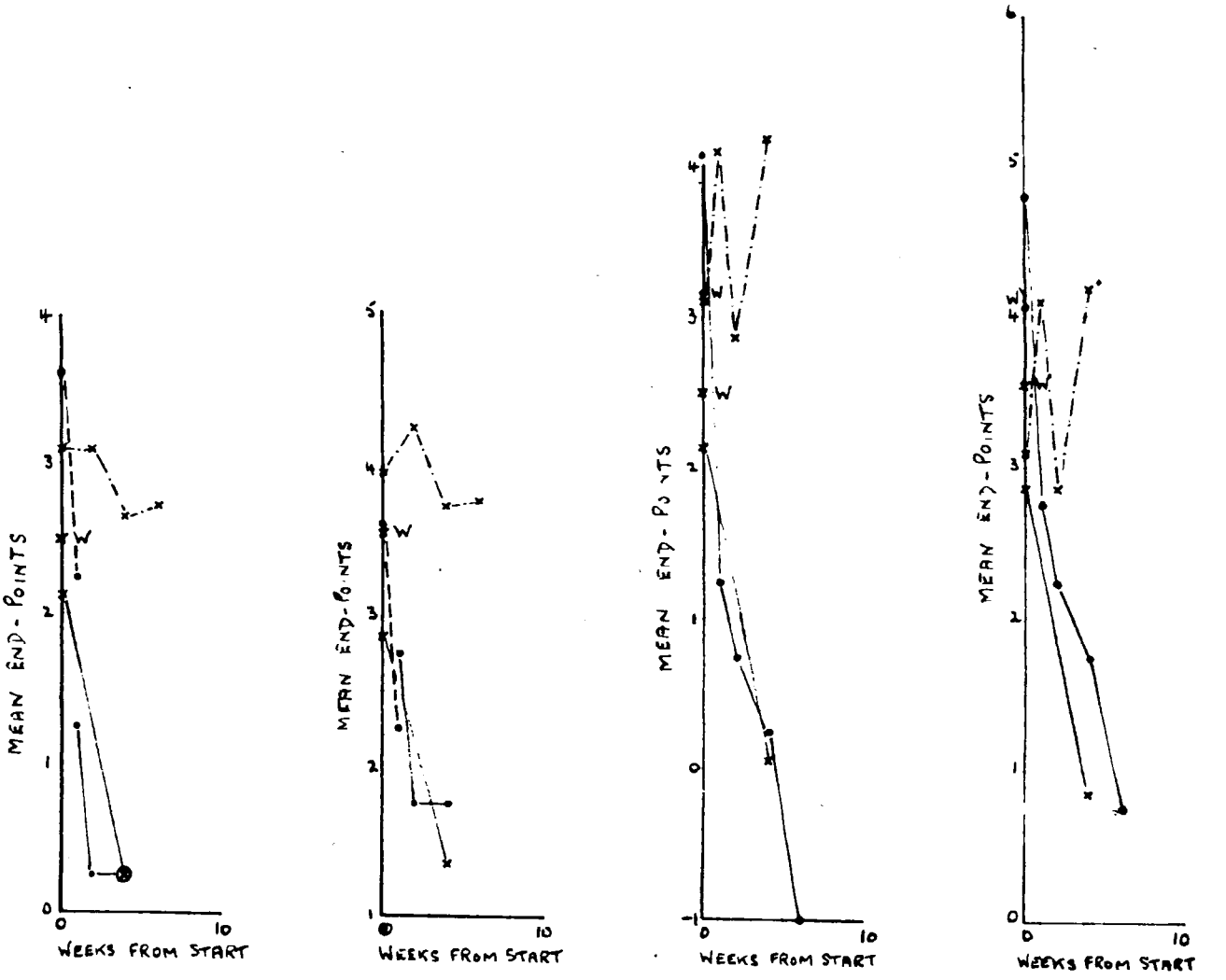



FIG. 1c.

Vaccine prepared at X

Mean End-points for Dry, Wet and Standard Vaccines - 0 - 4°C.

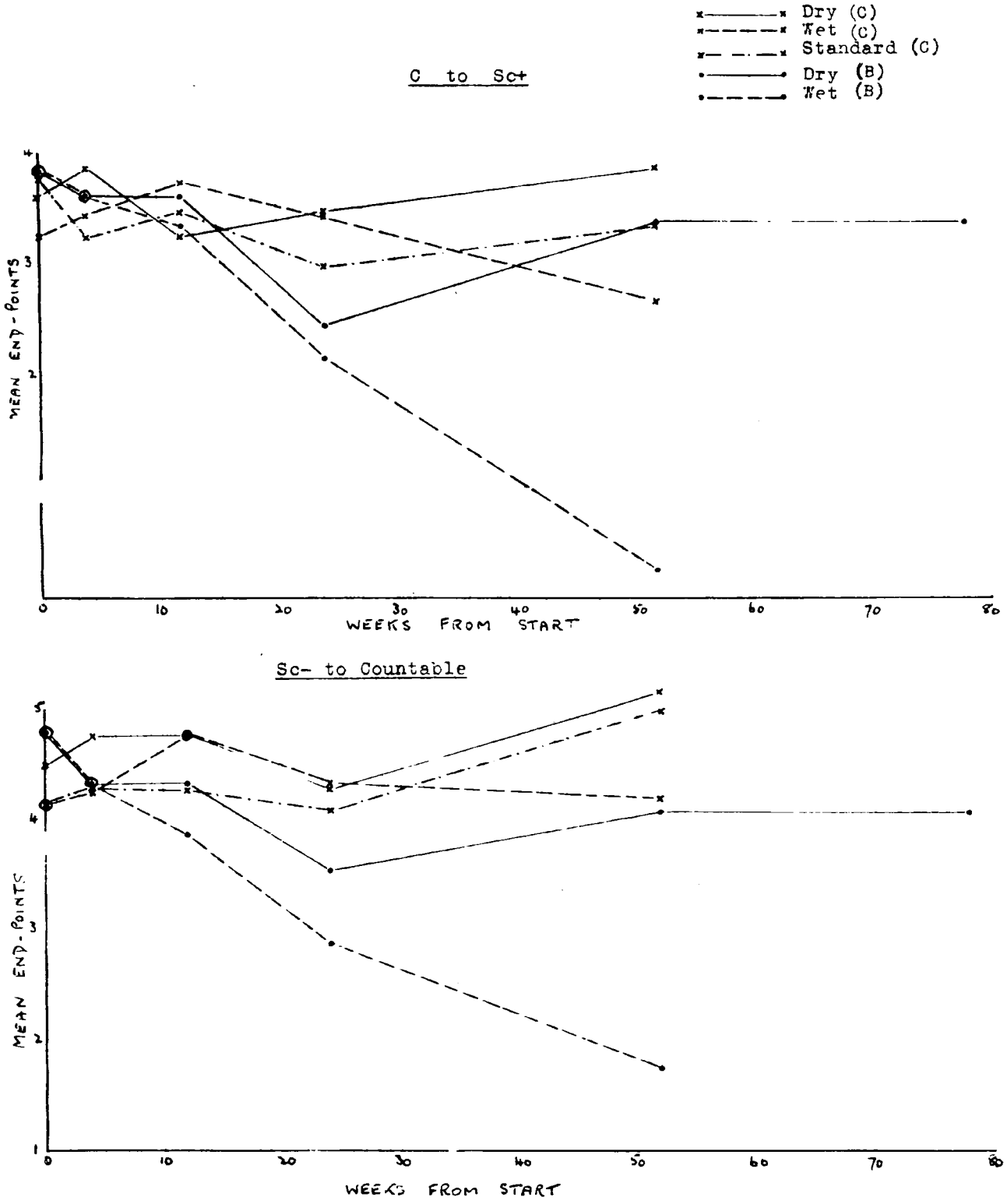



FIG. 2 a.

Vaccine prepared at X

Mean End-points for Dry, Wet and Standard Vaccines - 22°C.

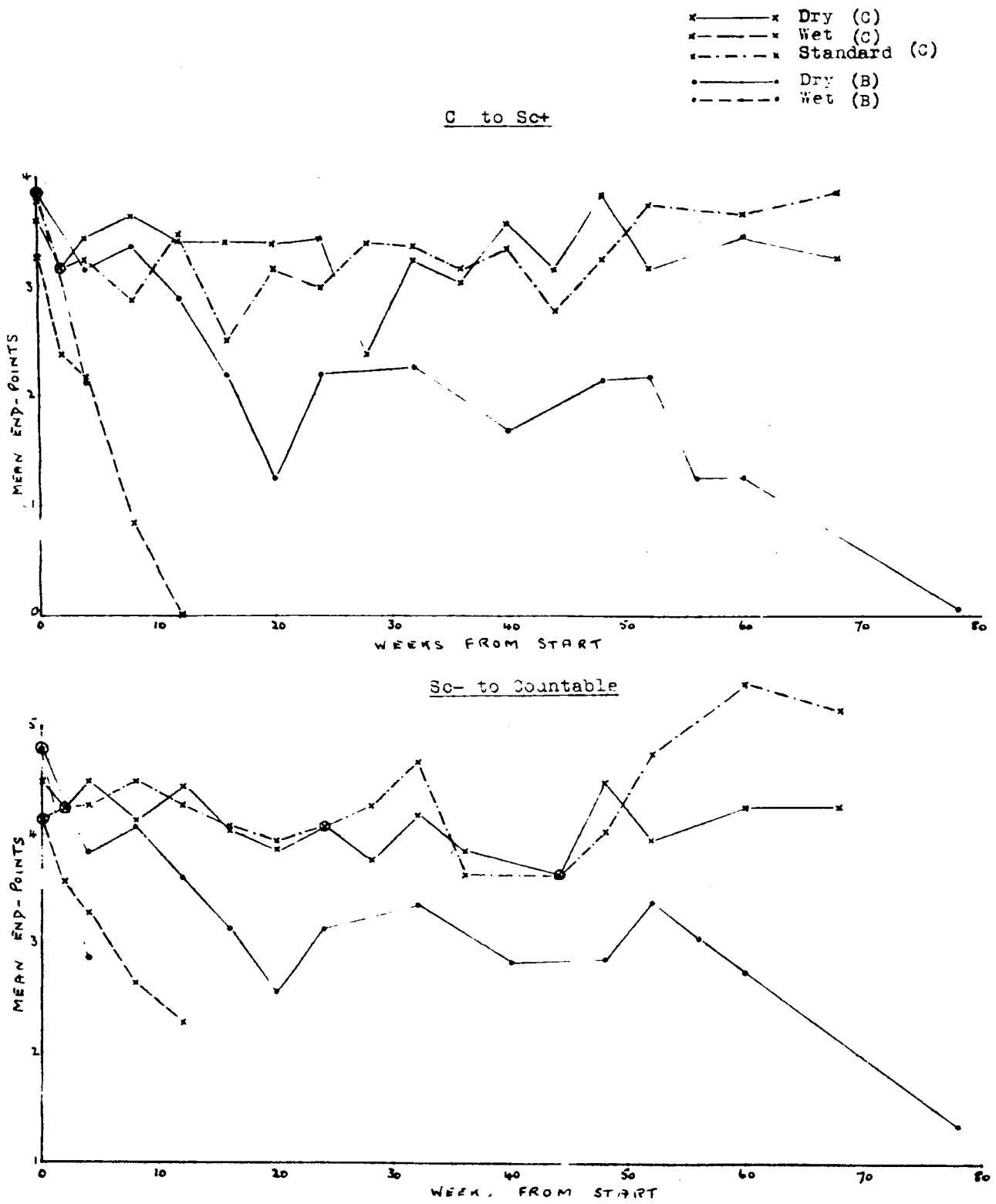
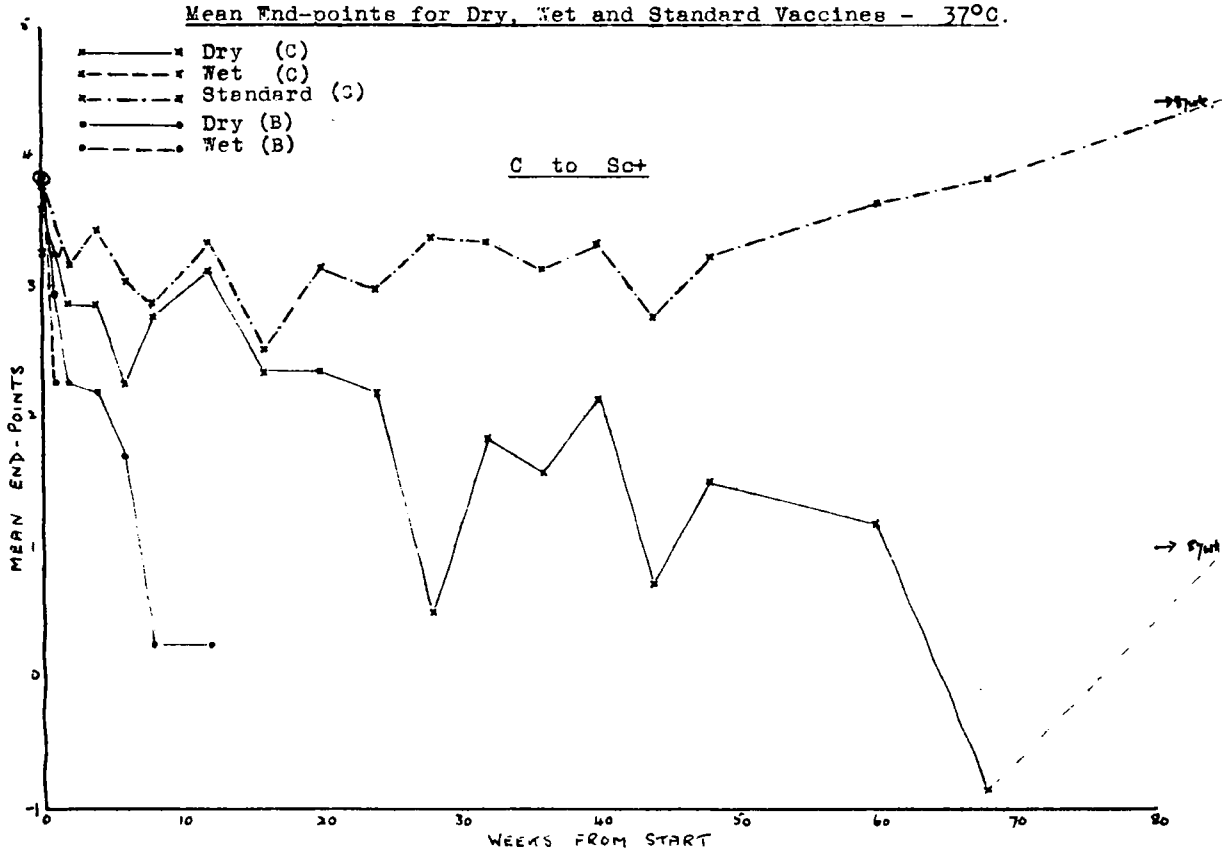



Fig. 2 b

Vaccine prepared at X

Mean End-points for Dry, Wet and Standard Vaccines - 37°C.




Sc- to Countable

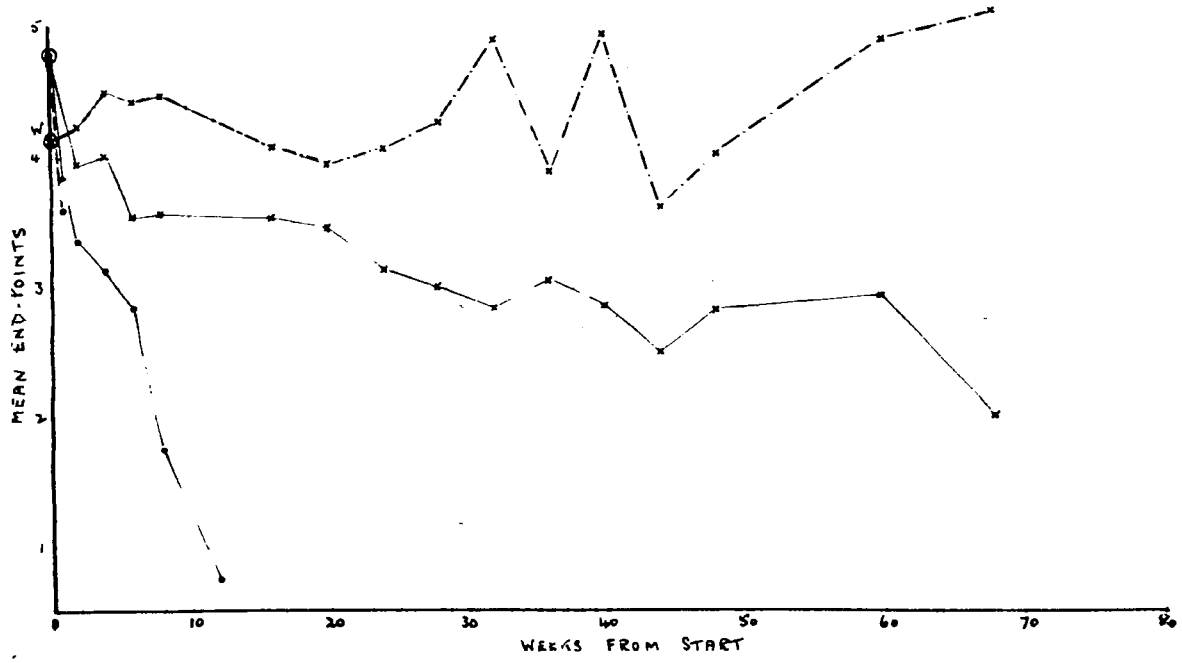


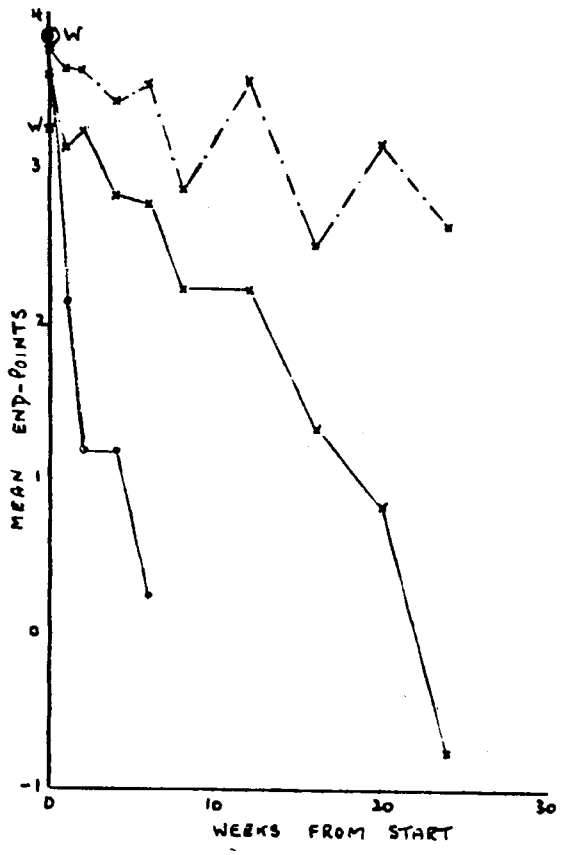

FIG. 2c.

Vaccine prepared at X

Mean End-points for Dry, Wet and Standard Vaccines - 45°C.

Dry (C)
Wet (C)
Standard (C)
Dry (B)
Wet (B)

C to Sc+




Sc- to Countable

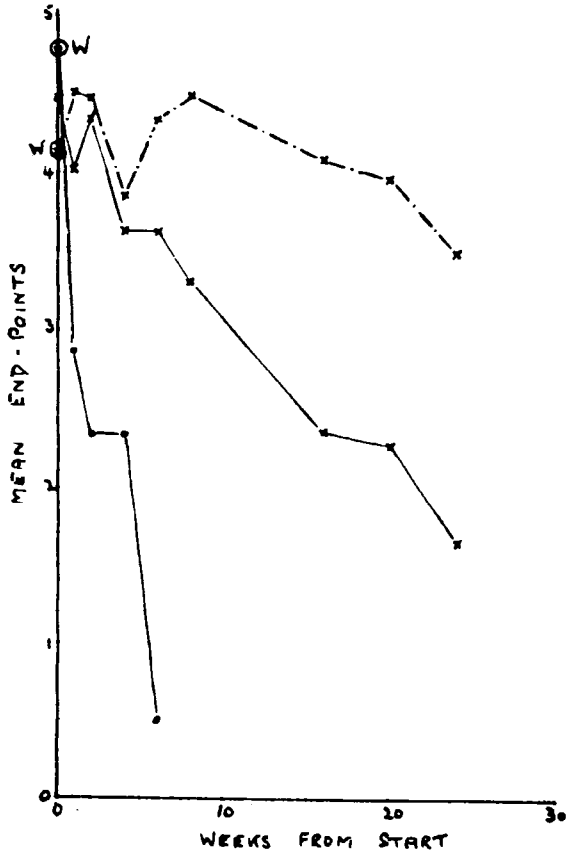
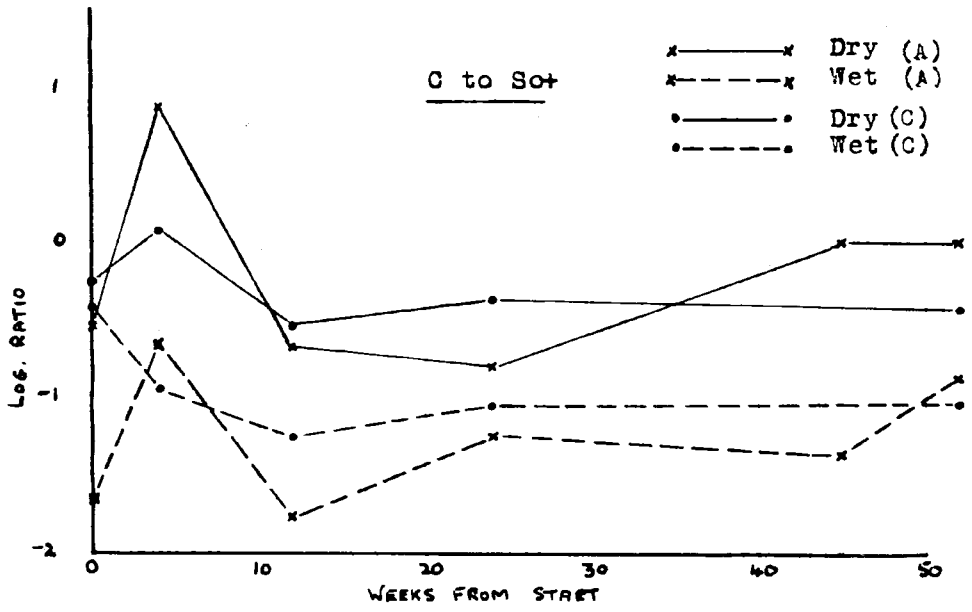



FIG. 2.d.

Vaccines prepared at Y

Estimates of Potency Ratios for Dry and Wet Vaccines - 0-4°C.




Sc- to Countable

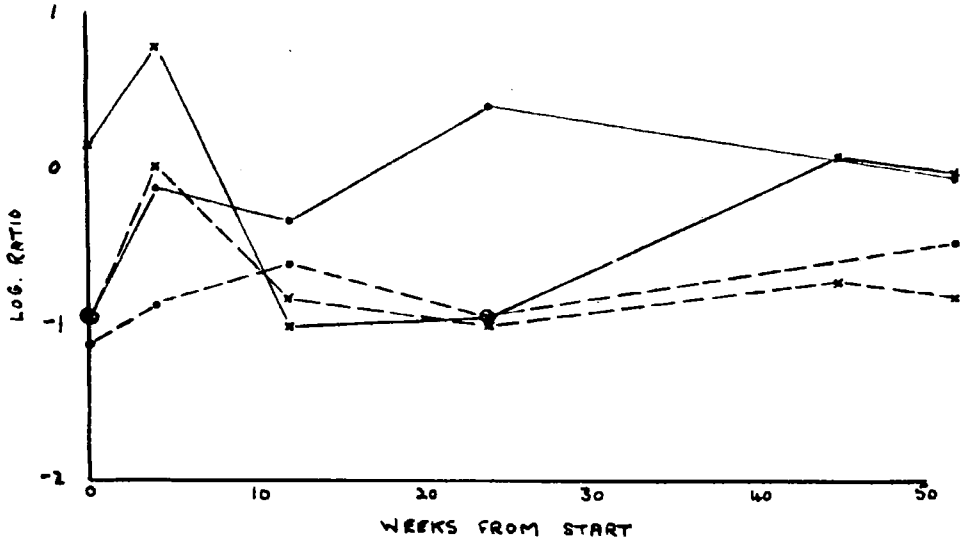
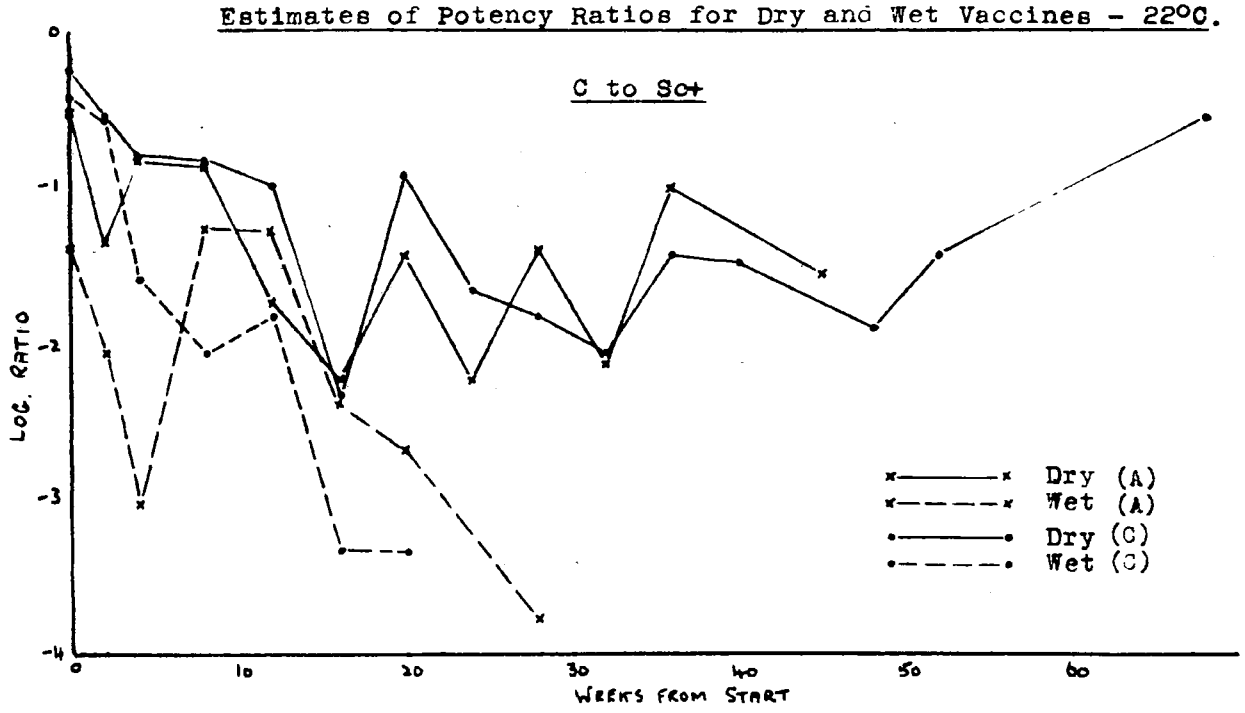



FIG. 3a.

Vaccines prepared at Y

Estimates of Potency Ratios for Dry and Wet Vaccines - 22°C.



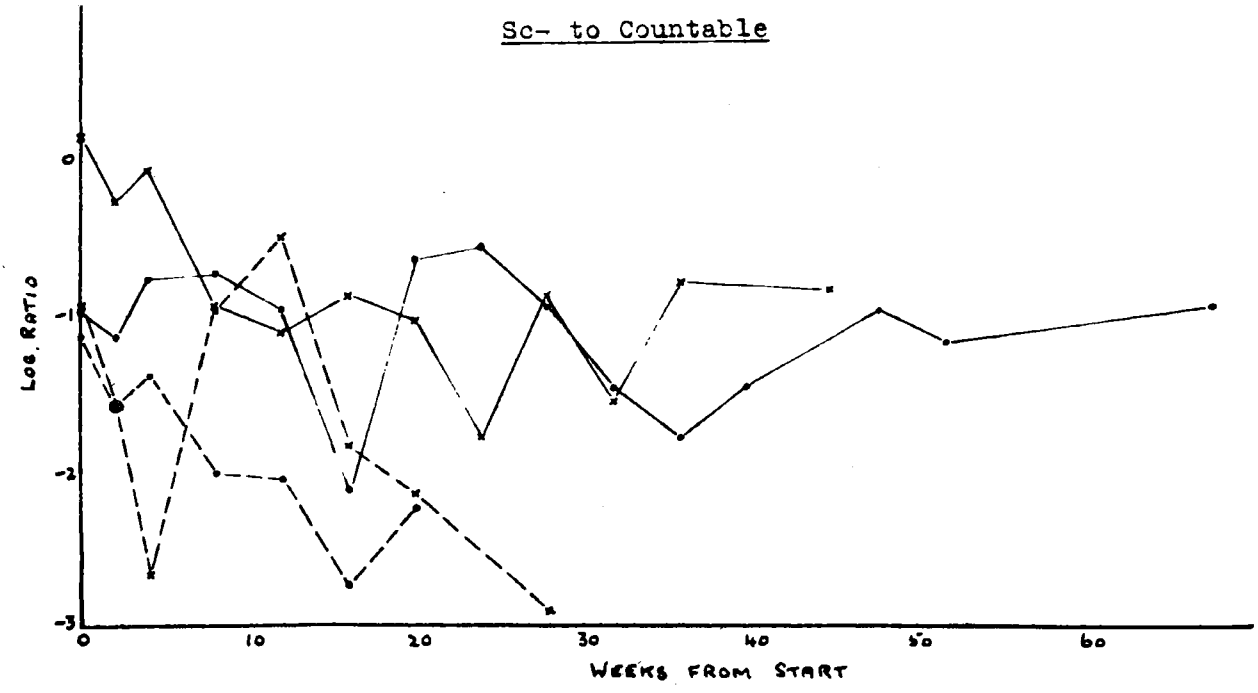




FIG. 3b

Vaccines prepared at Y

Estimates of Potency Ratios for Dry and Wet Vaccines - 37°C.

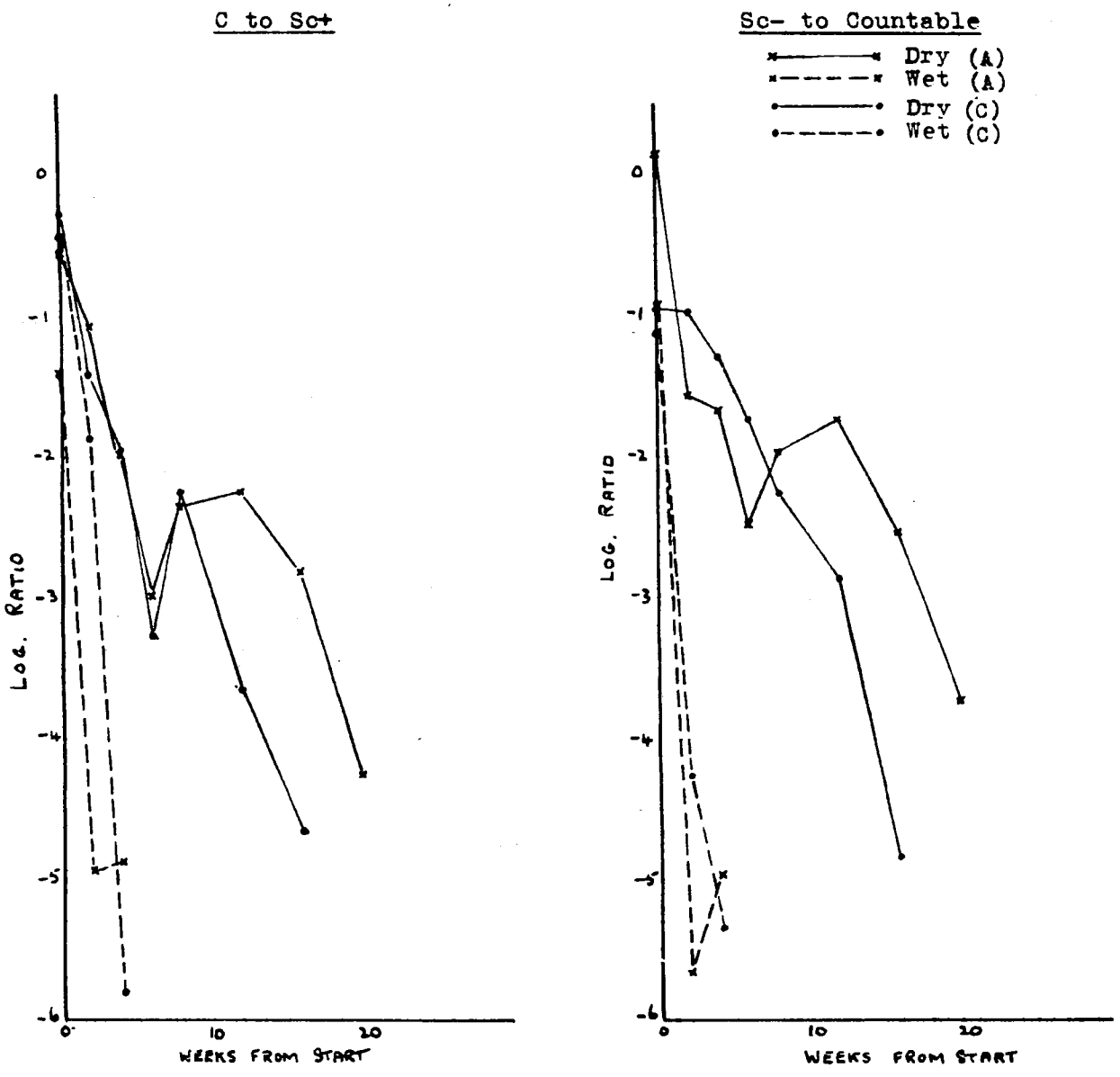



Fig. 3c.

Vaccines prepared at Y

Estimates of Potency Ratios for Dry and Wet Vaccines - 45°C.

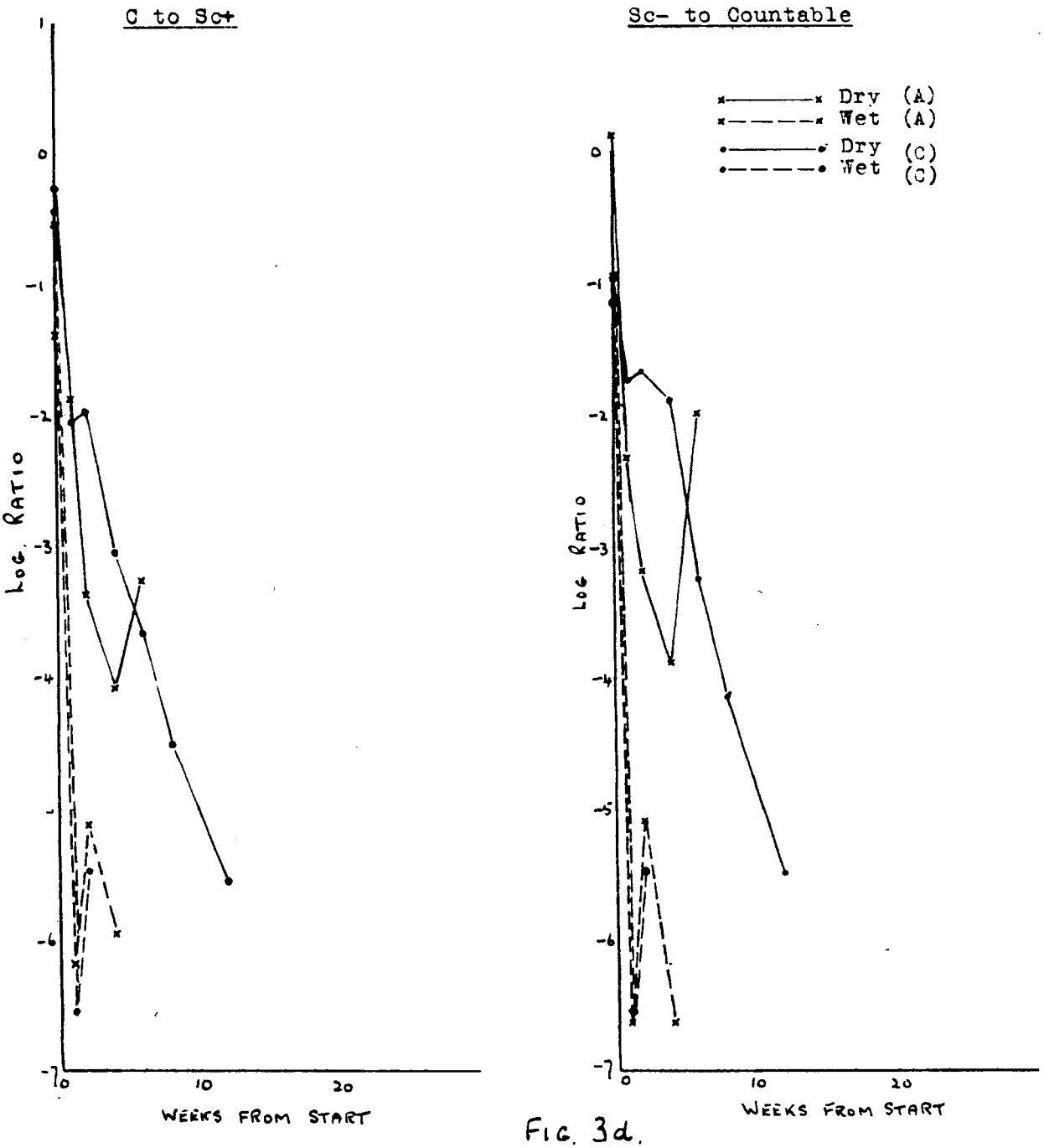



Fig. 3d.

Vaccine prepared at 2

Mean End-points for Dry, Wet and Standard Vaccines 0 - 4°C.

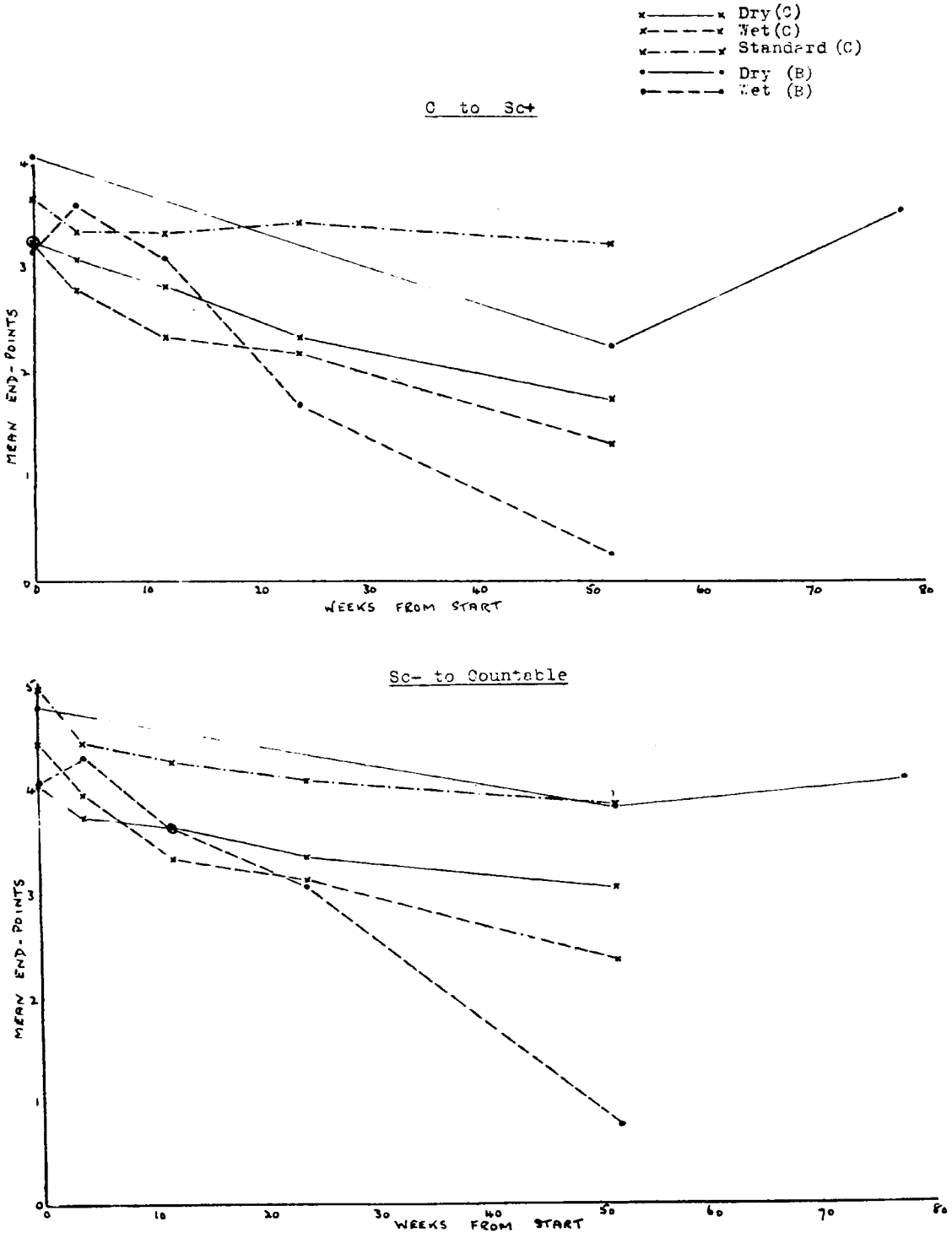



FIG. 4a.

Vaccine prepared at Z

Mean End-points for Dry, Wet and Standard Vaccines - 22°C.

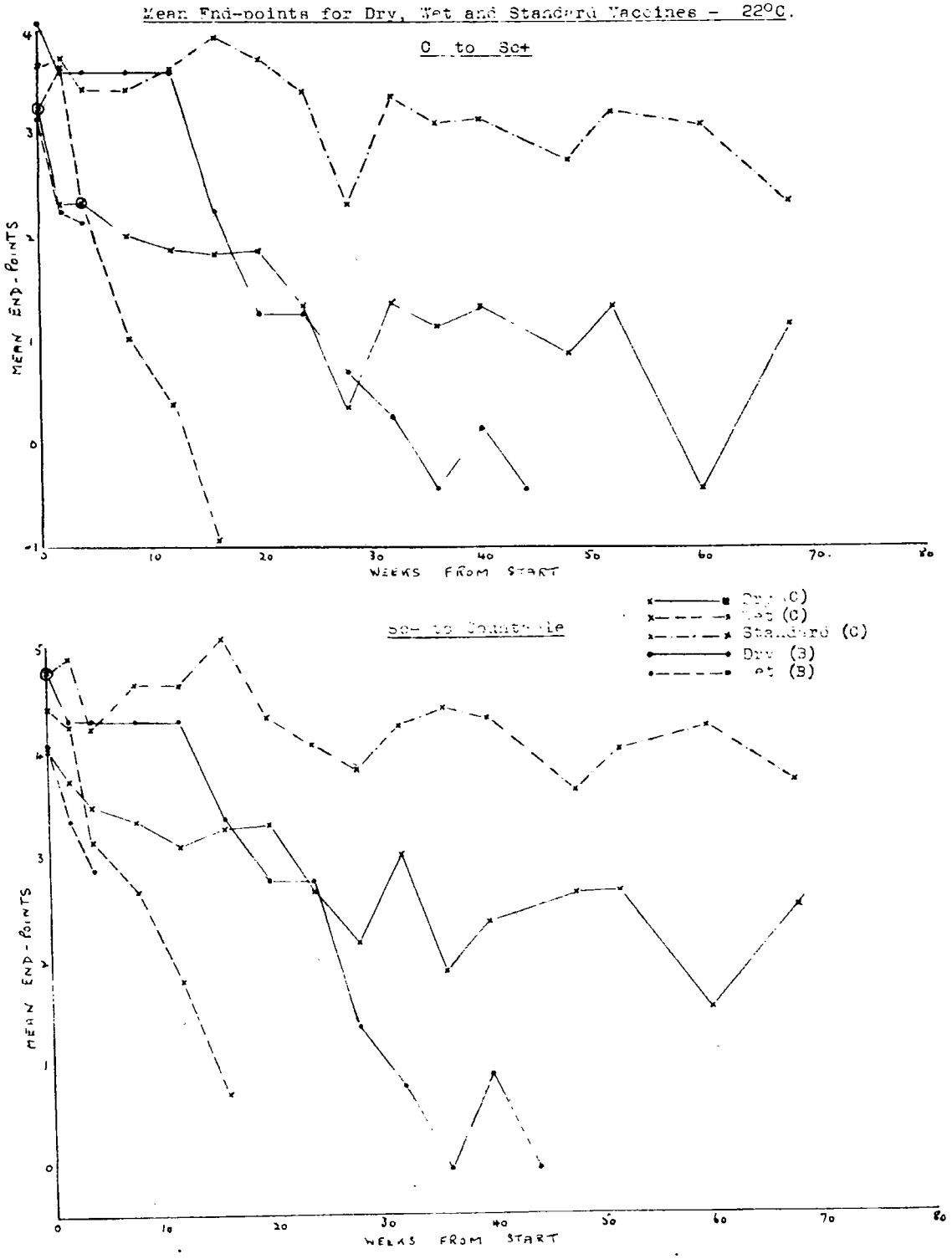


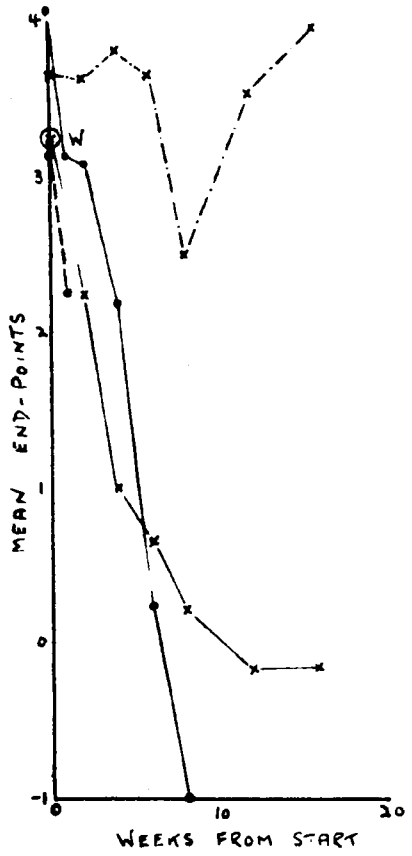

FIG. 4b.

Vaccine prepared at Z

Mean End-points for Dry, Wet and Standard Vaccines - 37°C.

Dry (C)
Wet (C)
Standard (C)
Dry (B)
Wet (B)

C to Sc+




Sc- to Countable

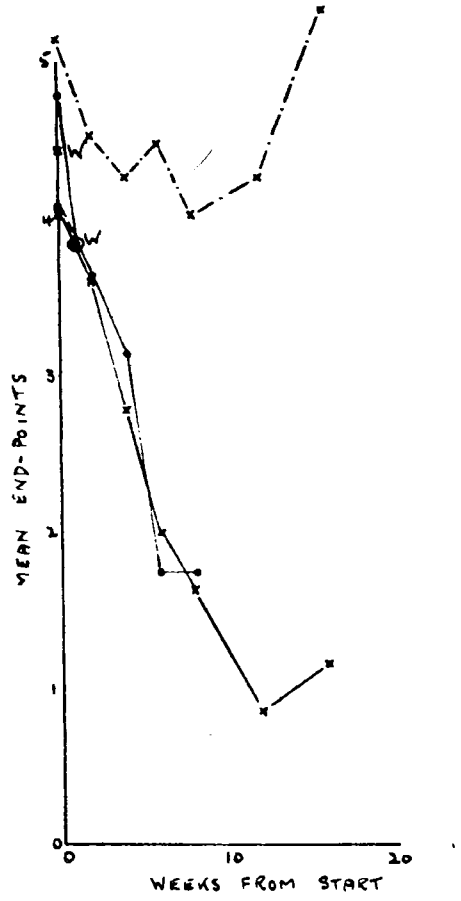


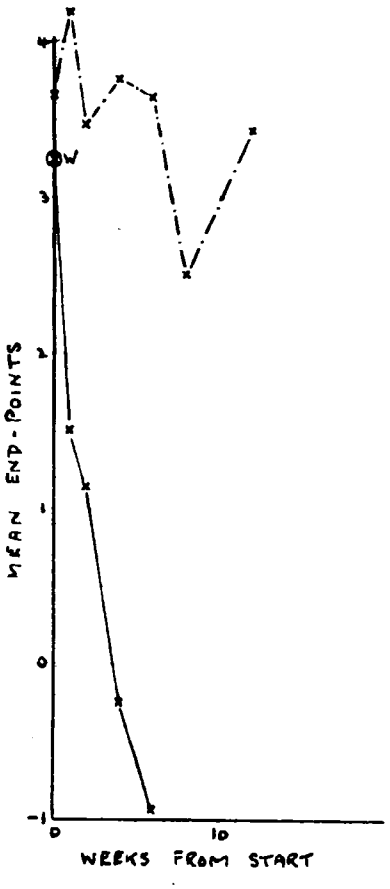

Fig. 4c.

Vaccine prepared at Z

Mean End-points for Dry, Wet and Standard Vaccines - 45°C.

Dry (C)
Wet (C)
Standard (C)

C to Sc+




Sc- to Countable

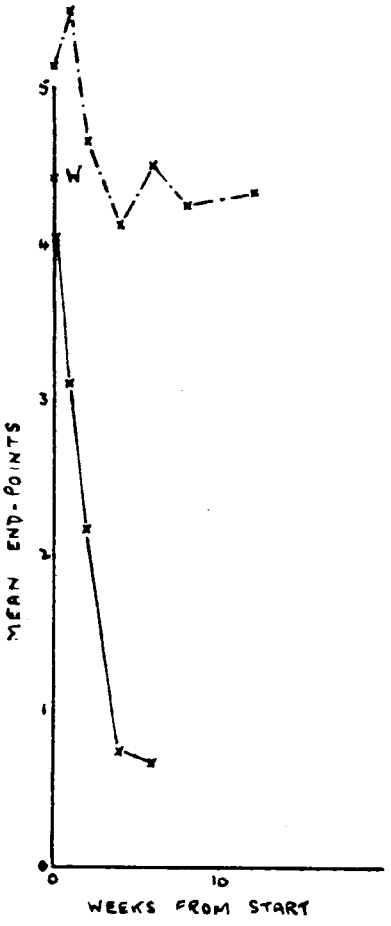



FIG. 4d.

# REPORT ON THE BACTERIAL PURITY OF THE VACCINES TESTED

by

Douglas McClean

The consultative group that designed the form of this trial of dried and glycerinated smallpox vaccines agreed that, to be acceptable for use, a vaccine should be free from anaerobic and aerobic pathogens and should not contain more than a total of 1000 bacteria per ml. It was also agreed not to lay down any standardized bacteriological technique but that each testing laboratory should employ methods that it used normally in the examination of smallpox vaccines. In addition to the total bacterial content of the vaccines, the tests should be designed to detect the presence of Cl. tetani, Bac. anthracis, β-haemolytic streptococci and Staphylococcus aureus. Any strain of Staph. aureus isolated that produced coagulase would be regarded as potentially pathogenic.

The absence of a standard technique makes it difficult to present the results obtained in a compact form, but the variety of methods used probably renders the general agreement obtained between laboratories the more convincing. The results obtained are set out in the Table (see p. 3).

Both the dried and glycerinated vaccines from X and Z were of entirely satisfactory purity; the total bacterial count was well below the permitted level and no potential pathogens were detected. The glycerinated vaccine from Y was also entirely satisfactory. No potential pathogens were detected in the dried vaccine from Y but the total count made in laboratory A was above the permitted level; the total count made at laboratory C (shown in the table) and confirmed by repetition gave an anomalous result which may be explained by the presence of un-neutralized antibiotics in the vaccine which masked the presence of viable bacteria. The glycerinated lymph from W gave a total count below the permitted level, but potential pathogens, both aerobic and anaerobic were detected in both laboratories A and B. The dried vaccine W was grossly contaminated with a total count of about 50 000 organisms per ml and these included many potential pathogens.

| W | X | Y | Z |
|---|---|---|---|
| Dried Vaccine<br>50 000 org./ml.<br>After 6 months' storage at 22° 600 org./ml.<br>Haem. Strep. Group C present.<br>Enterococci present.<br>No pathogenic anaerobes or Staph. pyogenes seen.<br>Glycerinated lymph<br>800 org./ml.<br>After 6 months' storage at 22° 100 org./ml.<br>Haem. strep. Group C present.<br>Enterococci present.<br>No pathogenic anaerobes or Staph. pyogenes seen. | | Dried Vaccine<br>5 000 - 6 000 org./ml.<br>After 6 months' storage at 22° 600 org./ml.<br>No pathogens seen.<br>Glycerinated lymph<br>100 org./ml.<br>After 6 months' storage at 22° 100 org./ml.<br>No pathogens seen. | |
| | Dried Vaccine<br><100 org./ml.<br>No E. coli, Haem. strep., Cl. tetani or B. anthracis seen.<br>One Staph. c- isolated in 0.01 ml. on blood agar.<br>Glycerinated lymph<br><100 org./ml.<br>No E. coli, Haem. strep. staph., Cl. tetani or B. anthracis seen. | Dried Vaccine<br>1 colony in 0.001 ml.<br>12 colonies in 0.0001 ml.*<br>11 of these were c-two staph. and 1 a motile anthracoid: not B. anthracis.<br>No E. coli, strep. or Cl. tetani seen.<br>Some colonies morphologically resembling actinomycetes present.<br>Glycerinated lymph<br><100 org./ml.<br>No E. coli, Haem. strep., staph., Cl. tetani or B. anthracis seen. | Dried Vaccine<br><100 org./ml.<br>No E. coli, strep., Cl. tetani or B. anthracis seen.<br>3 col.c+ staph. isolated in 0.01 ml. on blood agar.<br>Glycerinated lymph<br><100 org./ml.<br>No E. coli, Haem. strep., staph., Cl. tetani o B. anthracis seen. |
| Dried Vaccine<br>>10 000 org./ml.<br>Predominance of staph. aureus and albus. Many c+ strains.<br>Gas-producing and gelatine-liquefying anaerobes present. | Dried Vaccine<br><100 org./ml.<br>No living organisms seen. | | Dried Vaccine<br><100 org./ml.<br>A few colonies morphologically resembling actinomycetes seen. |

e: The increase in number of colonies with progressive dilution of the dried vaccine from Y suggests that bacterial growth in the lower dilutions may have been inhibted by traces of antibiot or antiseptic carried over with the vaccine. The vaccinial material had apparently been treated with an unspecified amount of penicillin and streptomycin; a higher bacterial count mig have been obtained if both these antibiotics could have been neutralized.

reviations:

| | | | | | |
|---|---|---|---|---|---|
| org./ml. | = | total organisms per ml. vaccine | c+ | = | coagulase positive |
| Haem. | = | haemolytic | c- | = | coagulase negative |
| Strep. | = | streptococci | < | = | less than |
| Staph. | = | staphylococci | > | = | more than |