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THE RESISTIVITY OF MICROORGANISMS TO INACTIVATION BY DRY HEAT

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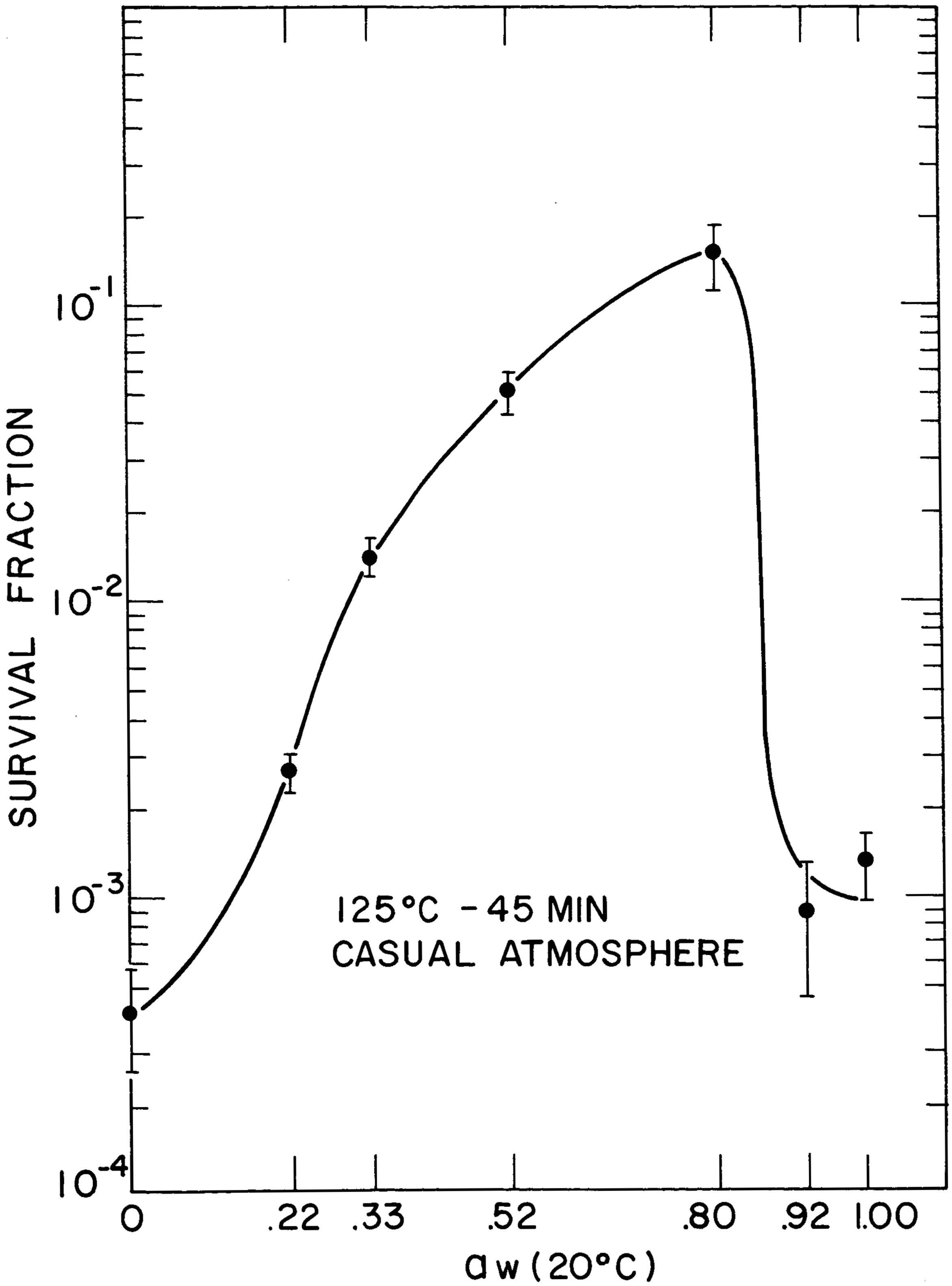
This report summarizes the data obtained in the past year on the importance of internal (IW) and external (EW) water in influencing the dry heat resistivity of *Bacillus subtilis* var. niger. The internal water content is considered to be established by equilibrating the spores over appropriate salt solutions at 20°C. to give water activities (A_W) of 0.0, 0.15, 0.22, 0.33, 0.52, 0.80, 0.92 and 1.0. External water is established by adding definite quantities of water to air passing over the spores. The levels of water contained by the air (heated to definite temperatures) were 0%, 1.8% and 20% relative humidity (RH) and the temperatures employed for both the air and the chamber were 115C, 125C and 135C. The support material upon which the spores were placed were glass cover slips. The air movement was either casual (convection) or had a velocity of 3 liters per minute.

RESULTS AND DISCUSSION

In agreement with previous work done in the investigator's laboratory and in other laboratories it is seen that there is an optimal internal water content which, for maximal resistance under these experimental conditions and for this organism, was approximately 0.80 (Fig. 1). This resistance was maximal at A_W=0.80 for a variety of spore crops, three of which are presented in Fig. 2. It is interesting to note that wide variations occurred at the low moisture content but that the spore crops all optimized at 0.80. When flowing air was employed, Fig. 3 at low RH of 1.8% the optimal A_w was still 0.80 but at higher RH (20%) maximal resistance occurred over the entire A_w range of 0 to 0.80 but at A_w's greater than 0.80 resistance decreased. It therefore appears that EW is extremely important and capable of overcoming an apparent deficiency of IW. This protection afforded by water is extremely rapid, Fig. 4, 5 and 6, and water does not appear to alter the shape of the inactivation curve. Inactivation is exponential and the magnitude of the protective effect of water is greater for the drier spores. This latter fact is illustrated in Figure 7. In addition, these figures emphasize the importance of EW, as measured by the distance between the essentially parallel inactivation curves. The effectiveness of the protective effect of water is in part also a function of temperature (Fig. 8) and EW and at approximately 138°C and 20% RH the influence of IW is eliminated. In order for moisture to be effective it must be present at the same time that the spores are exposed to thermal inactivation (Fig. 12). There is little or no repair mechanisms evident, for if there were the inactivation curves for spores exposed to dry air and then to 1.8% RH would parallel that of spores exposed to 1.8% RH and then to dry air.

CONCLUSION

It must be concluded that both external and internal water is protective but that the degree of effectiveness depends upon temperature and the extent of A_W. At A_W's greater than 0.80 or at temperatures higher than 130°C water loses its effectiveness. In agreement with others, the spore appears to be freely permeable to water, even at elevated temperatures. These experiments do not give one insight into the location or the composition of the heat susceptible target in the spore. The action of the target though is such that it must either be hygroscopic or be surrounded by hygroscopic material. This hydrogroscopicity is effective at elevated temperatures and stabilizes the target.



SURVIVAL FRACTION
10-1
10-2
10-3
10-4
125°C - 45 MIN
CASUAL ATMOSPHERE
0
.22
.33
.52
.80
.92
1.00
aw (20°C)

FIG. 1

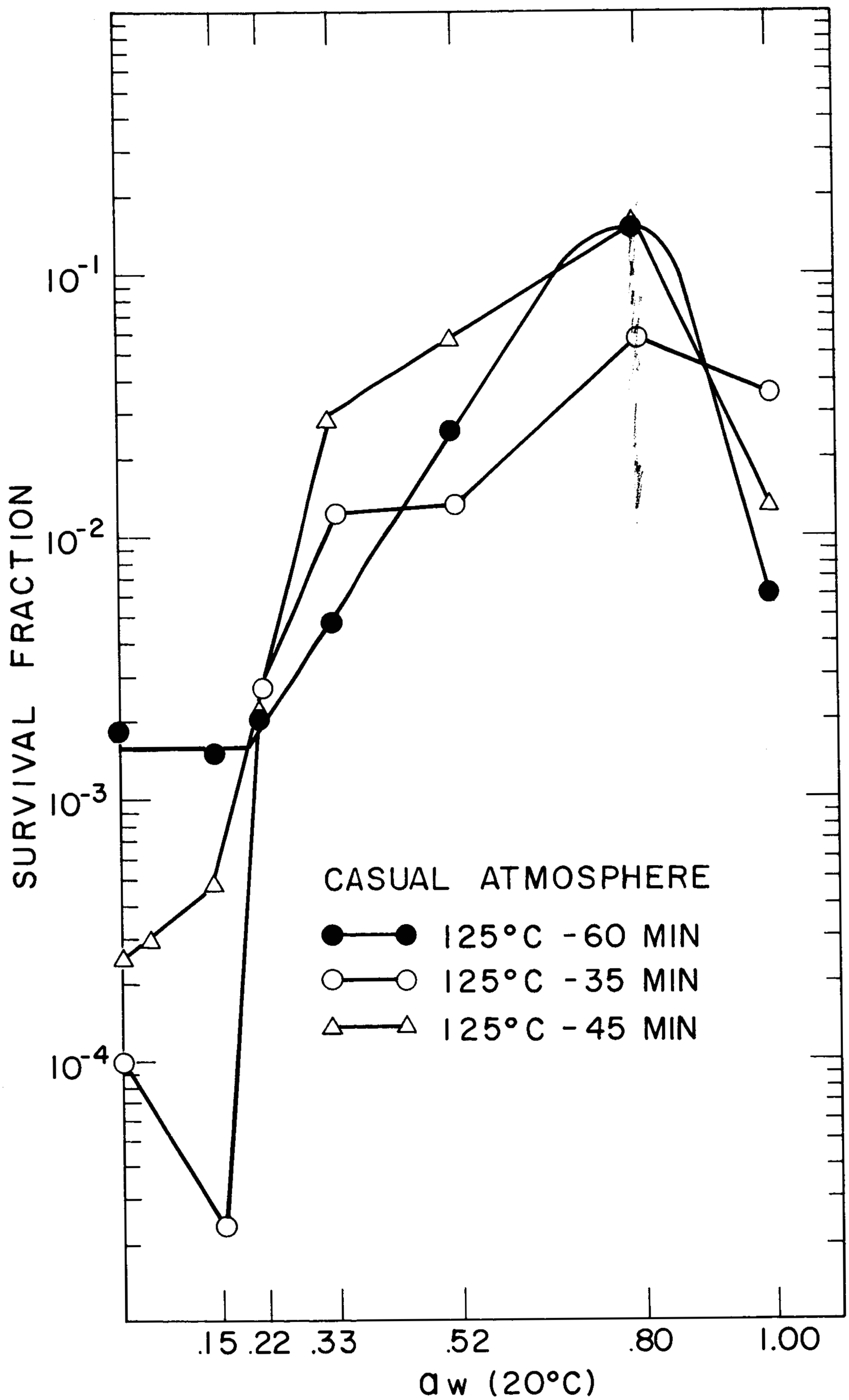

SURVIVAL FRACTION
10^{-1}
10^{-2}
10^{-3}
10^{-4}
CASUAL ATMOSPHERE
125°C -60 MIN
125°C -35 MIN
125°C -45 MIN
.15
.22
.33
.52
.80
1.00
aw (20°C)

FIG. 2

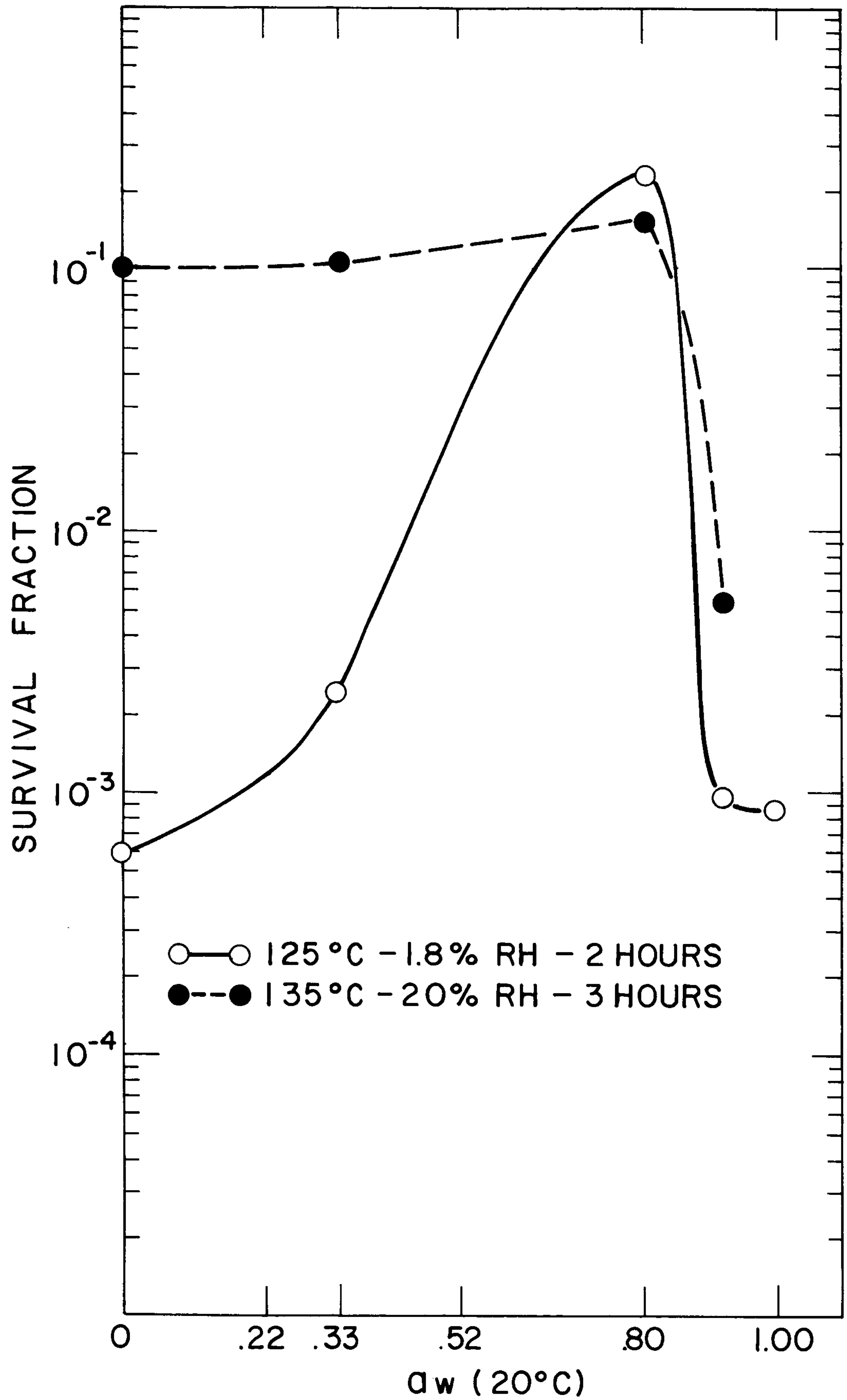

SURVIVAL FRACTION
10^{-1}
10^{-2}
10^{-3}
10^{-4}
125 °C – 1.8 % RH – 2 HOURS
135 °C – 20 % RH – 3 HOURS
0
.22
.33
.52
.80
1.00
aw (20°C)

FIG. 3

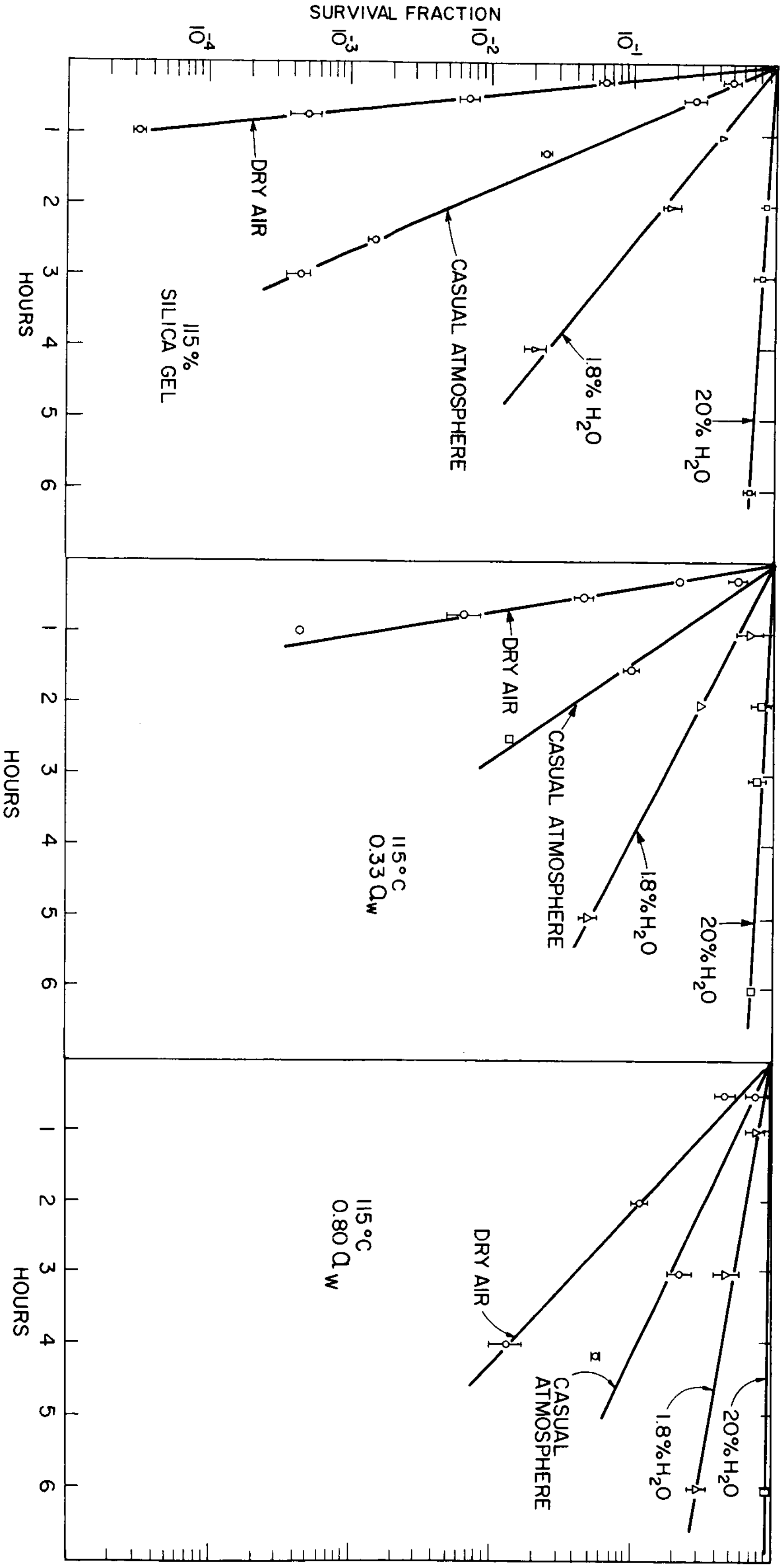

SURVIVAL FRACTION
10^{-1}
10^{-2}
10^{-3}
10^{-4}
HOURS
DRY AIR
CASUAL ATMOSPHERE
1.8% H_2O
20% H_2O
115%
SILICA GEL
115°C
0.33 a_w
0.80 a_w

FIG. 4

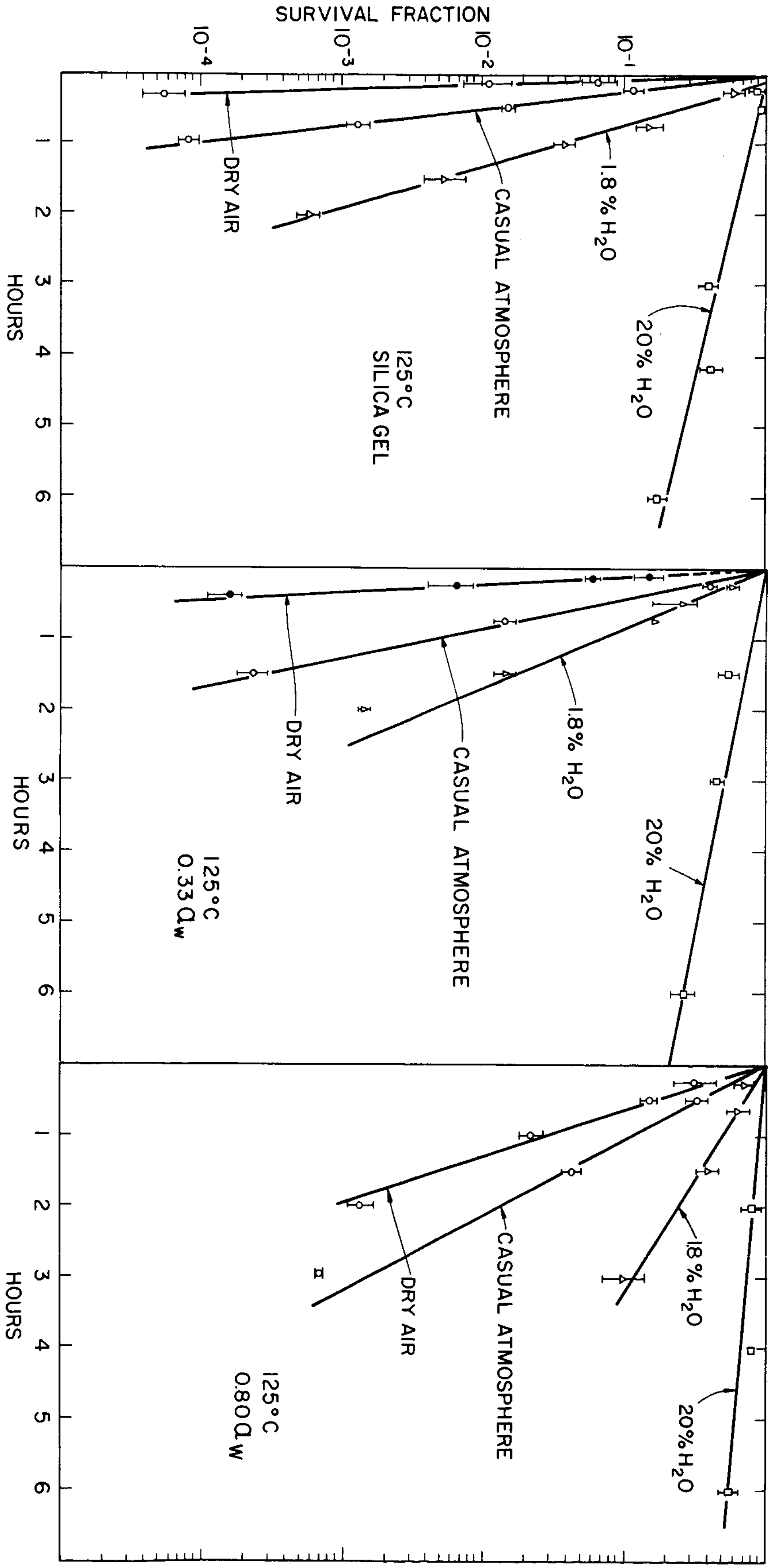
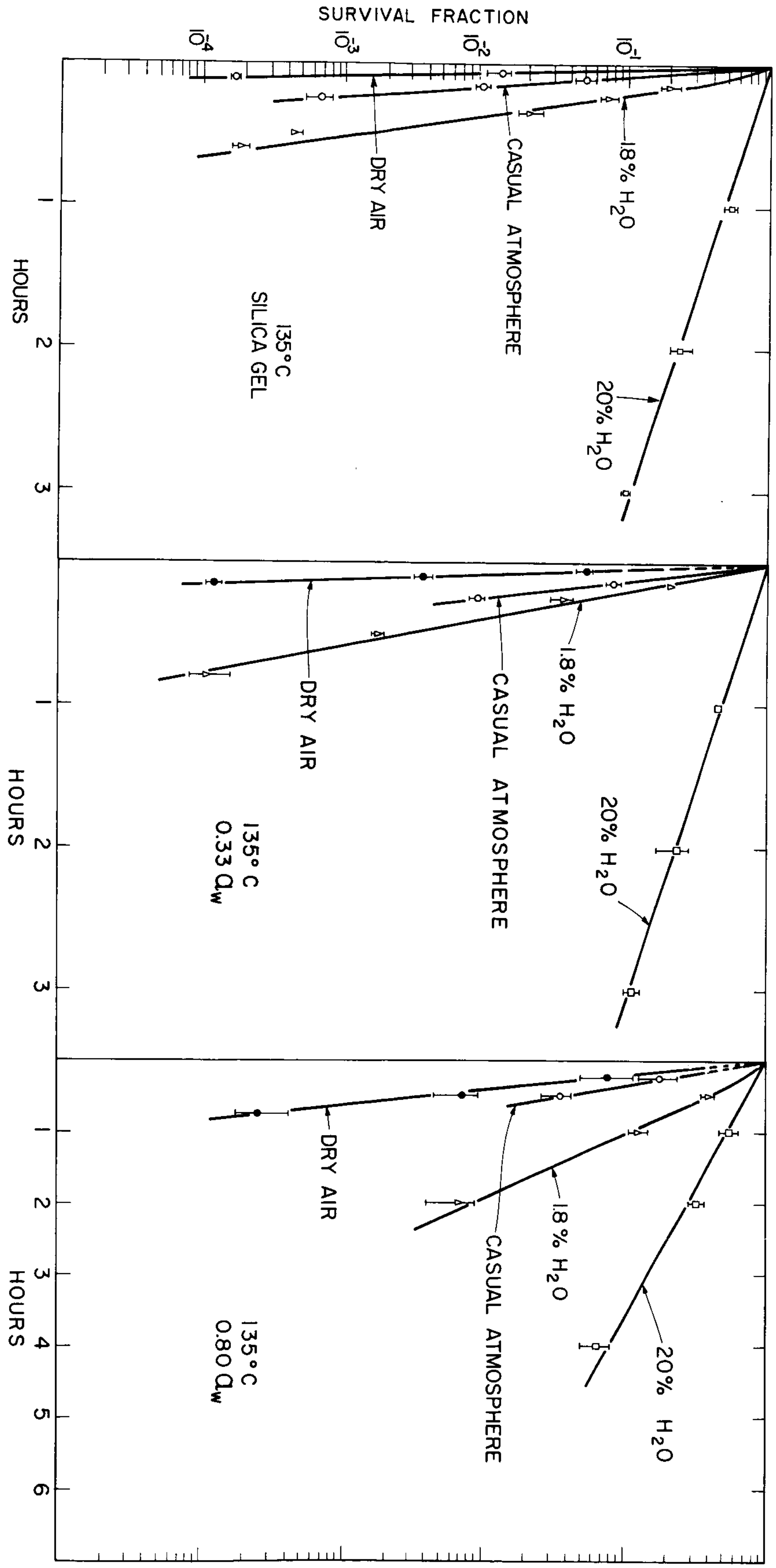

SURVIVAL FRACTION
10-1
10-2
10-3
10-4
HOURS
DRY AIR
CASUAL ATMOSPHERE
1.8% H_2O
20% H_2O
125°C
SILICA GEL
125°C
0.33 a_w
18% H_2O
125°C
0.80 a_w

FIG. 5



SURVIVAL FRACTION
10^{-1}
10^{-2}
10^{-3}
10^{-4}
HOURS
1
2
3
4
5
6
1.8% H_2O
20% H_2O
CASUAL ATMOSPHERE
DRY AIR
135°C
SILICA GEL
135°C
0.33 a_w
135°C
0.80 a_w

FIG. 6

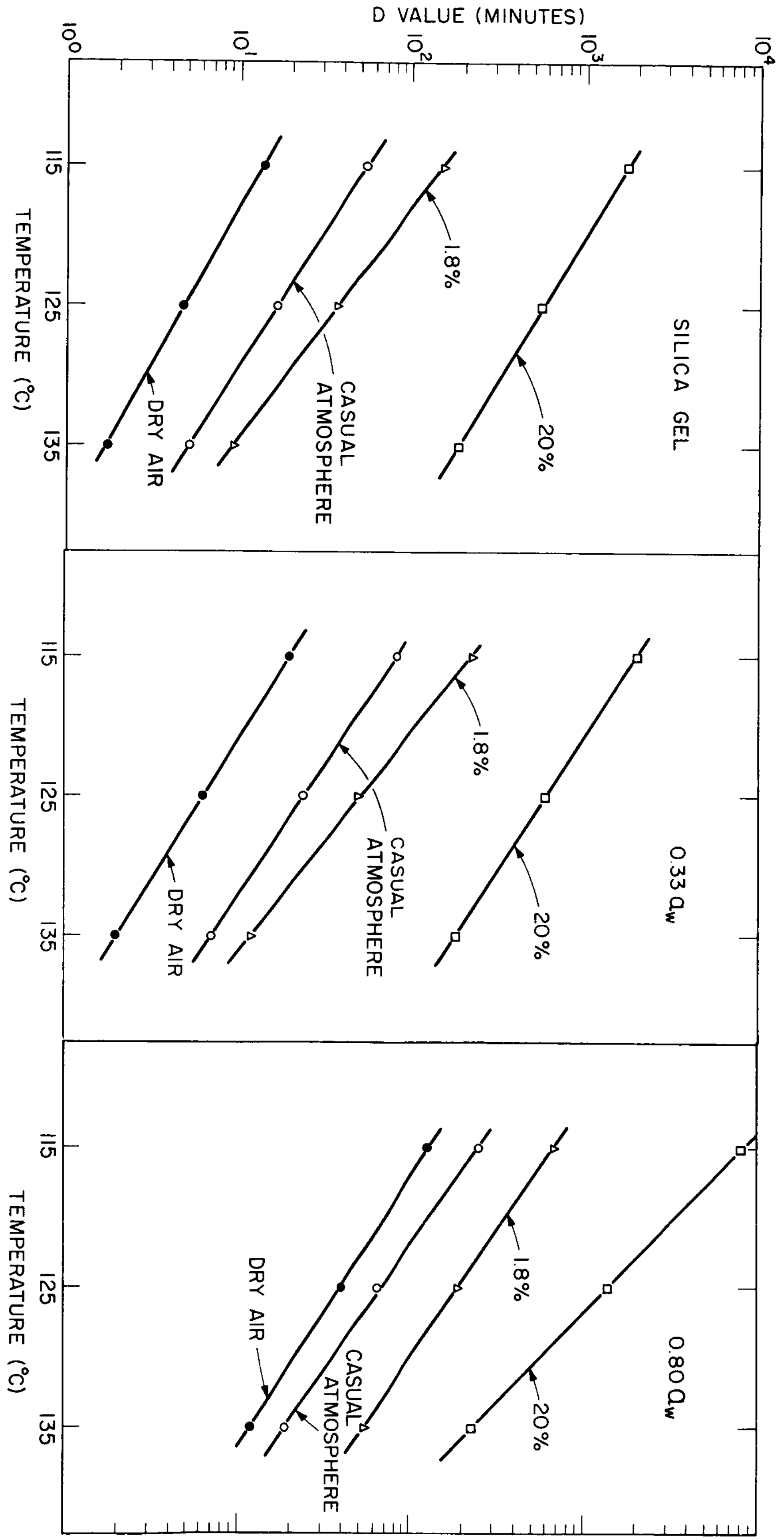

D VALUE (MINUTES)
10^0
10^1
10^2
10^3
10^4
SILICA GEL
0.33 a_w
0.80 a_w
20%
1.8%
CASUAL ATMOSPHERE
DRY AIR
115
125
135
TEMPERATURE (°C)

FIG. 7

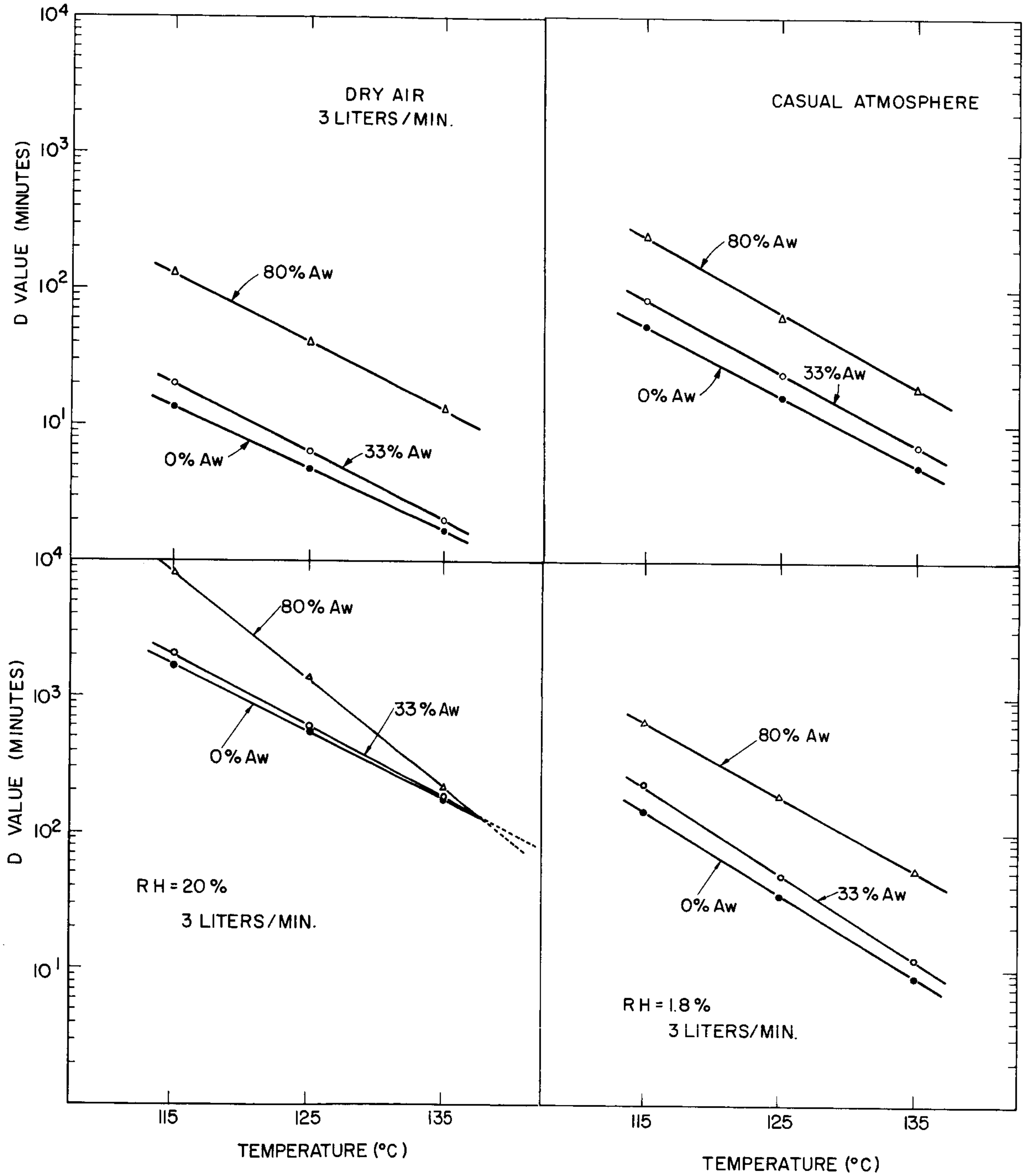

DRY AIR
3 LITERS/MIN.
CASUAL ATMOSPHERE
80% Aw
33% Aw
0% Aw
RH = 20%
3 LITERS/MIN.
RH = 1.8%
3 LITERS/MIN.
D VALUE (MINUTES)
TEMPERATURE (°C)
10^4
10^3
10^2
10^1
115
125
135

FIG. 8

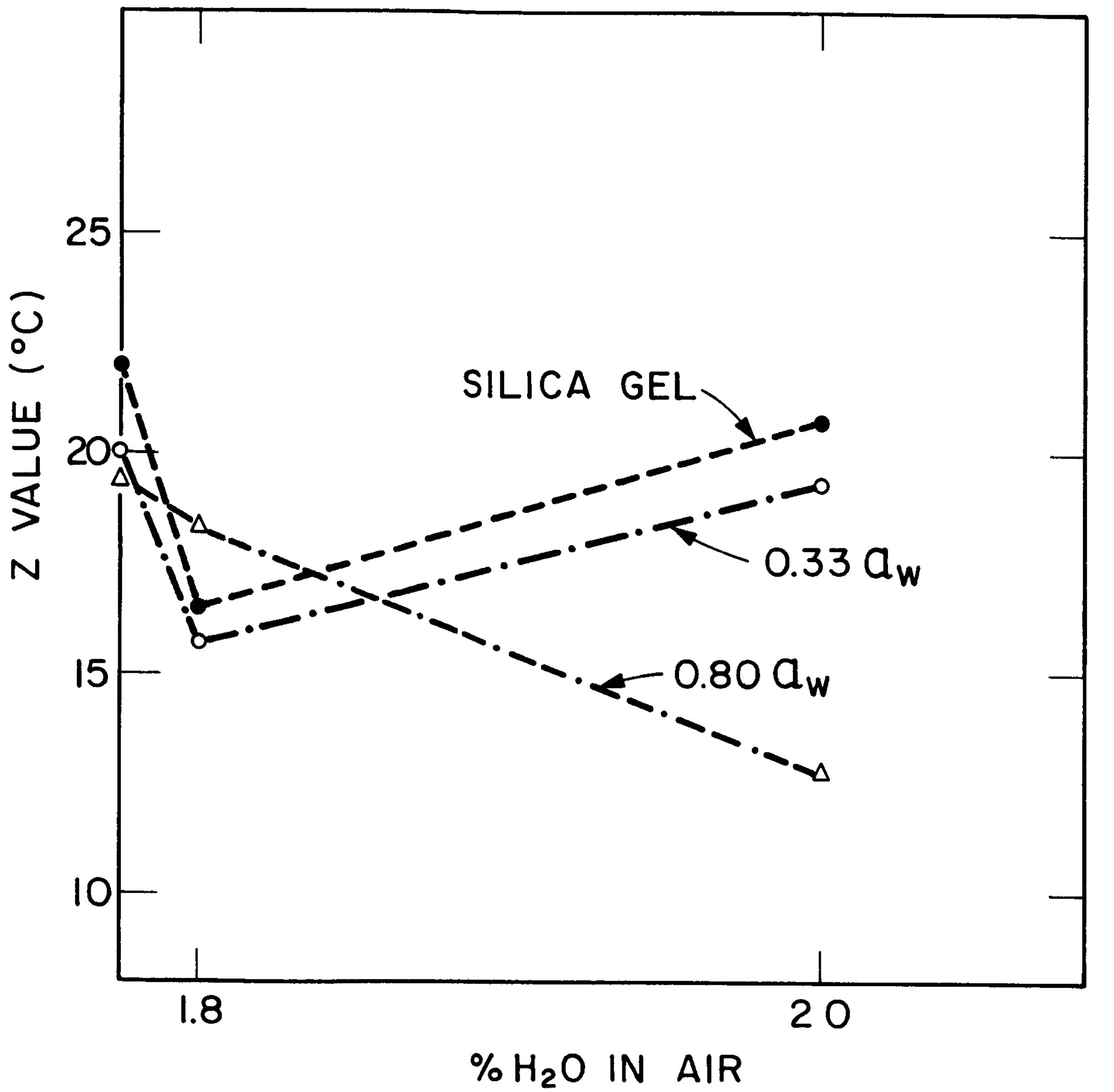


FIG. 9

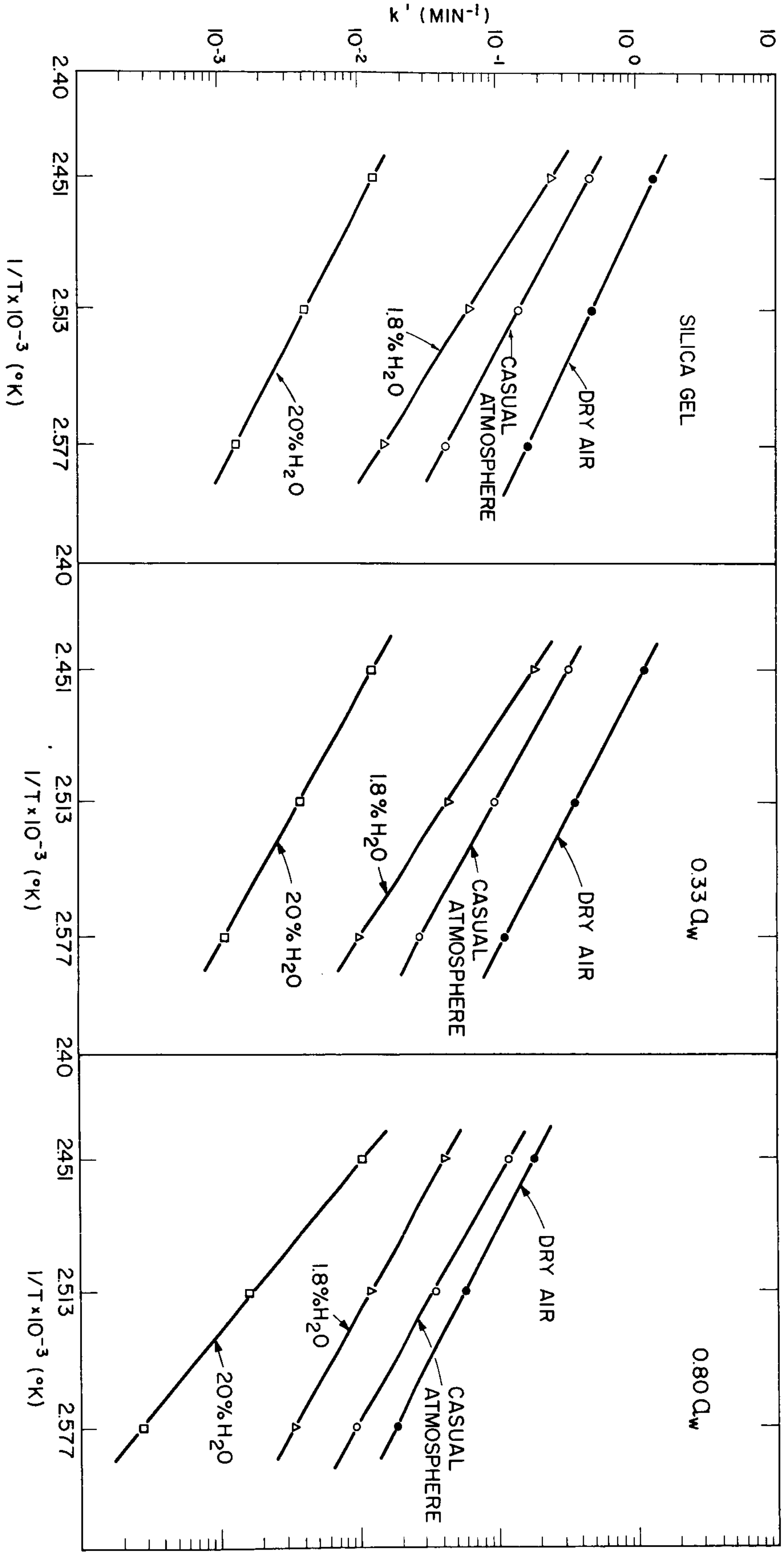

k' (MIN⁻¹)
10
10⁰
10⁻¹
10⁻²
10⁻³
2.40
2.451
2.513
2.577
1/T x 10⁻³ (°K)
SILICA GEL
0.33 a_w
0.80 a_w
DRY AIR
CASUAL ATMOSPHERE
1.8% H_2O
20% H_2O

FIG. 10

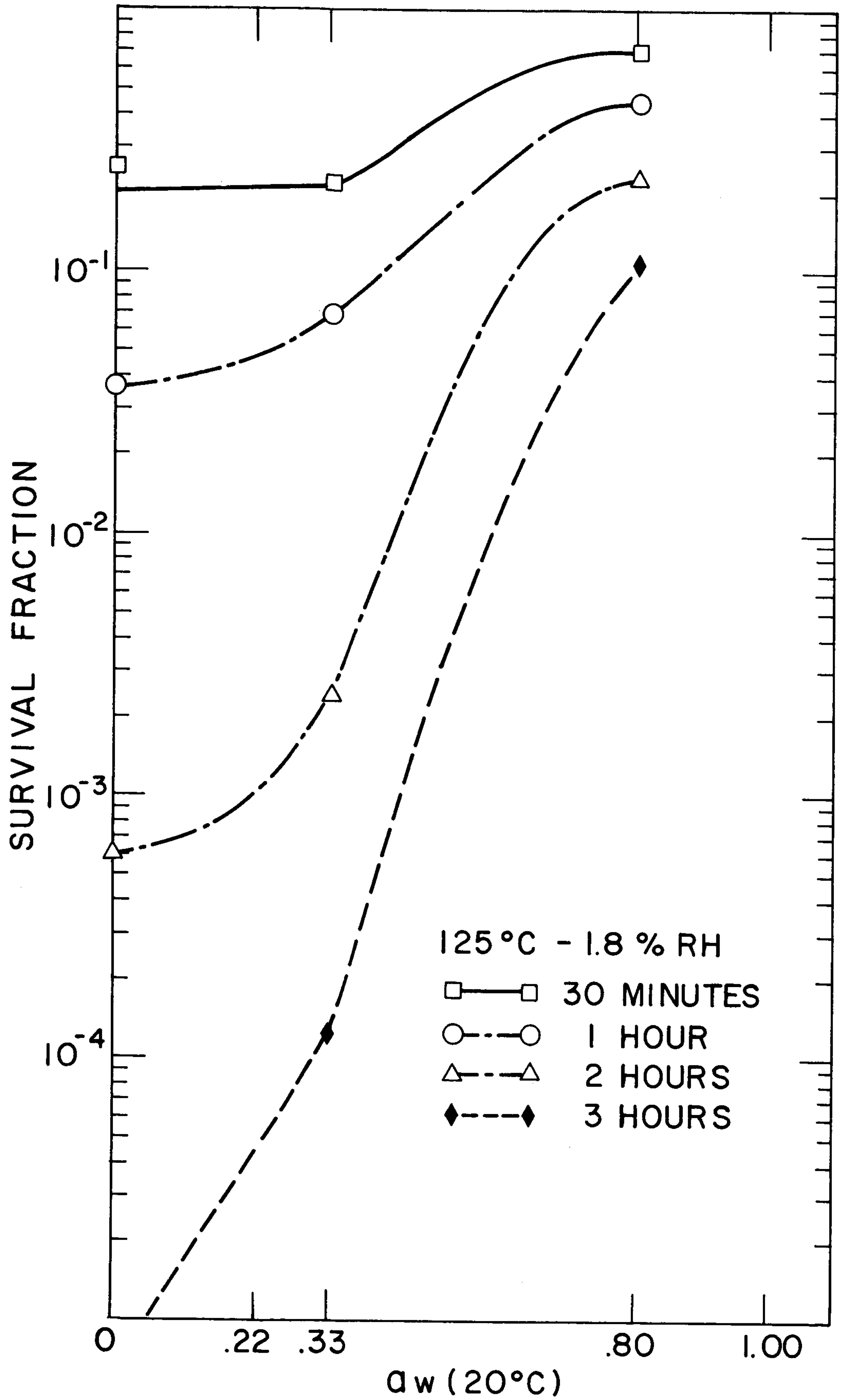
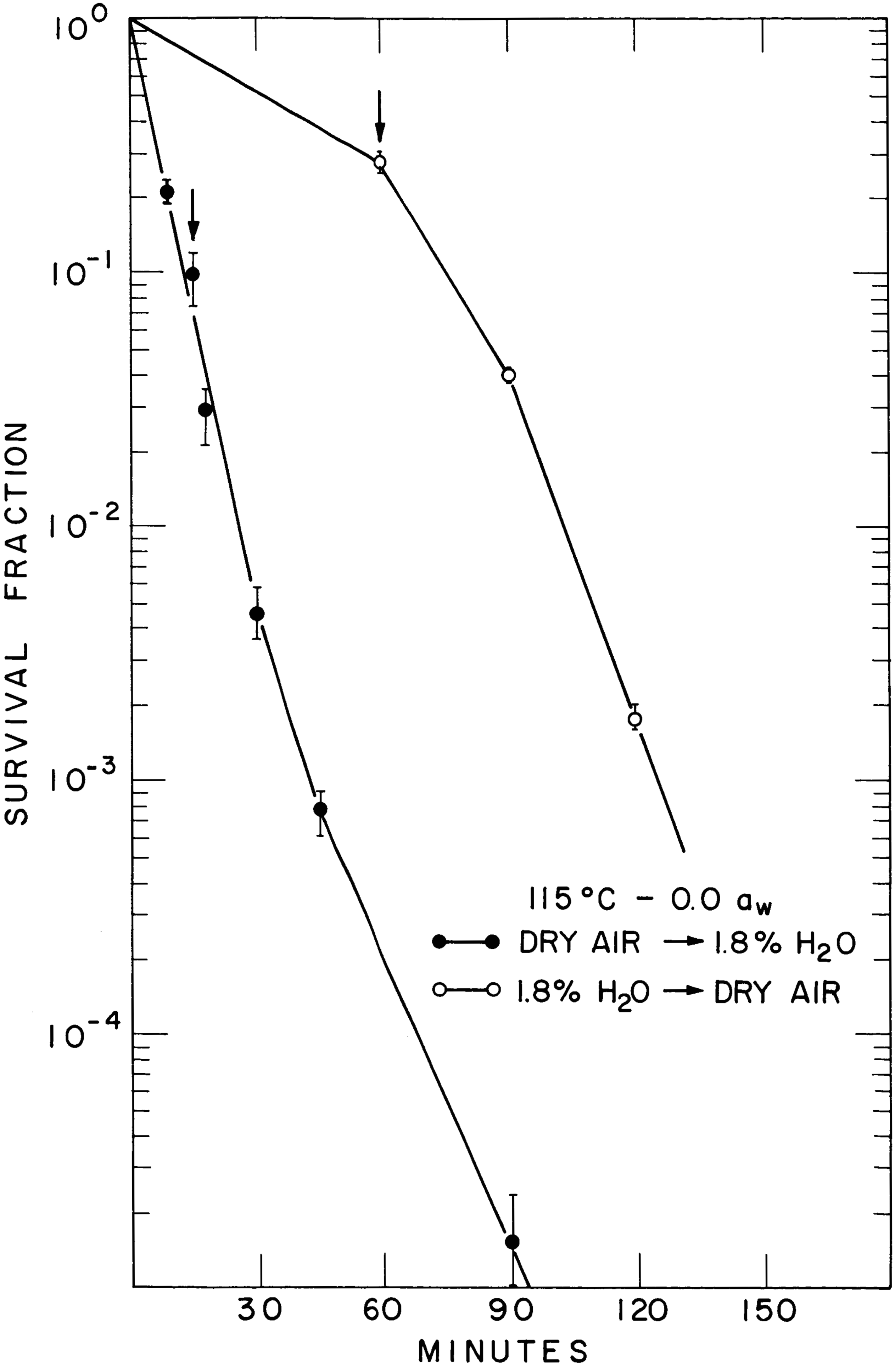

SURVIVAL FRACTION
10^{-1}
10^{-2}
10^{-3}
10^{-4}
125°C - 1.8 % RH
30 MINUTES
1 HOUR
2 HOURS
3 HOURS
0
.22
.33
.80
1.00
aw (20°C)

FIG. 11



10^0
10^{-1}
10^{-2}
10^{-3}
10^{-4}
SURVIVAL FRACTION
30
60
90
120
150
MINUTES
115 °C - 0.0 a_w
DRY AIR → 1.8% H_2O
1.8% H_2O → DRY AIR

FIG. 12