THE INFLUENCE OF TEMPERATURE ON THE RATE OF GROWTH OF *SPOROTRICHUM CARNIS*, FROM −10° C. TO +30° C.

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(With Six Text-figures.)

This mould is of frequent occurrence on stored carcases and in cold stores. The lean portions of carcases which have been kept for eight or ten weeks below zero centigrade but above − 10° C. are frequently covered with mould growth. No such growth has been so far observed on carcases stored at − 20° C. Brooks and Hansford, in reviewing mould growth on cold-store meat, found that some strains of *Cladosporium herbarum* developed and grew at − 6° C. (Brooks and Hansford, 1923). They also suggested that other moulds including *Sporotrichum*, would probably grow between 0° C. and − 6° C. It is therefore of interest to attempt to determine the precise growth limits of this mould. Two cases have to be investigated: the influence of temperature upon growth *per se*, and also the effect of physical changes in the medium consequent upon freezing. The present communication is concerned with the first of these only, all measurements below zero having been made on *supercooled* media: *i.e.* media cooled below their freezing-points but from which ice crystals had not separated.

EXPERIMENTAL.

The particular culture used was isolated from a cold store. Several platings, on a modified Czapek's agar, yielded a pure culture. This agar, which was used throughout in the growth experiments, had the following composition (Waksman and Curtis, 1916):

$NaNO_3$	2 gm.	Sucrose 30 gm.
K_2HPO_4	1 gm.	Distilled water 1 litre.
$MgSO_4\,7H_2O$	0·5 gm.	*p*H 6·4 approximately
KCl	0·5 gm.	measured colorimetrically.
$FeSO_4\,7H_2O$	0·01 gm.	

The stock culture was kept on agar slants of this medium. In general the spores were taken from a culture at least a week old.

A small quantity of sterile melted Czapek's agar was allowed to solidify on the centre of a sterile cover-slip in a sterile covered Petri dish. Care had to be exercised not to make the drop of agar of too great a thickness, or it would have been impossible to focus on its surface with the $\frac{1}{6}$ in. objective used. A little of the culture to be studied was then transferred with a sterile platinum wire to the agar and spread as evenly as possible. Cover-slip and agar were next pressed down on the top of a small glass ring of suitable size, cemented to a microscope slide with Canada balsam. The top ground surface of this ring had previously been coated with a stiff rubber grease which did not "run" at the temperature of incubation. A single large drop of water was placed in the centre of the floor of the chamber so formed to maintain a saturated atmosphere, Tomkins having shown the importance of humidity in mould growth (Tomkins, 1929) (see Fig. 1).

In many cases the special facilities of the Low Temperature Station permitted the carrying out of incubation and measurement in the same chamber, maintained at the desired constant temperature, so that no errors were introduced by the fluctuation of temperature caused by transport from incubator to microscope.

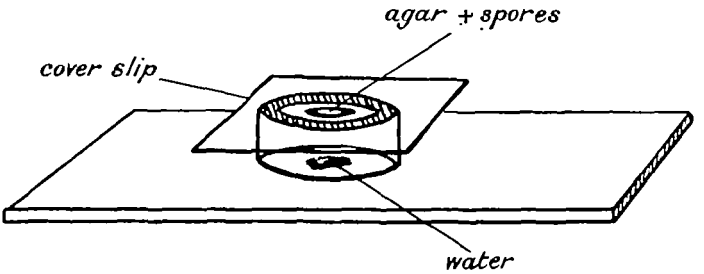

Fig. 1.

With the higher temperatures, however, the ordinary laboratory incubator was used and observation carried out as quickly as possible on removal to the microscope stage. Strictly speaking, measurement of the rate of growth should be a measurement of increased volume. If, however, observations are restricted to the early phases of growth and not made at the extreme temperatures at which the organism will grow, the diameter of the germ-tube is approximately constant. No error is thus introduced in substituting length for volume. This holds true only during the logarithmic phase of growth. Observations on older cultures showed that the threads were often several times as thick as in the earlier phase.

During normal growth the spores at first thicken to about twice their previous diameter, then elongate in one direction, a germ-tube being put out. In some cases another germ-tube makes its appearance in the same straight line as the first but in the opposite direction. More than two such germ-tubes have never been observed. Branching begins to take place some hours after germination at the higher temperatures. At 30° C. spores were seen to germinate and the threads to increase in length for a time, but the threads were thicker than those grown at more favourable temperatures. After some few hours a great deal of thickening and contortion of

the threads took place, so that most bizarre shapes were often produced (see Fig. 6). In this case the substitution of length for volume does not hold, and the growth at 30° C. cannot be estimated accurately. At − 5° C. the threads were slightly thicker than at more favourable temperatures, but slow extended growth without further thickening took place for about 2000 hours. After this, extension of the threads took place but comparatively slowly, more thickening and branching

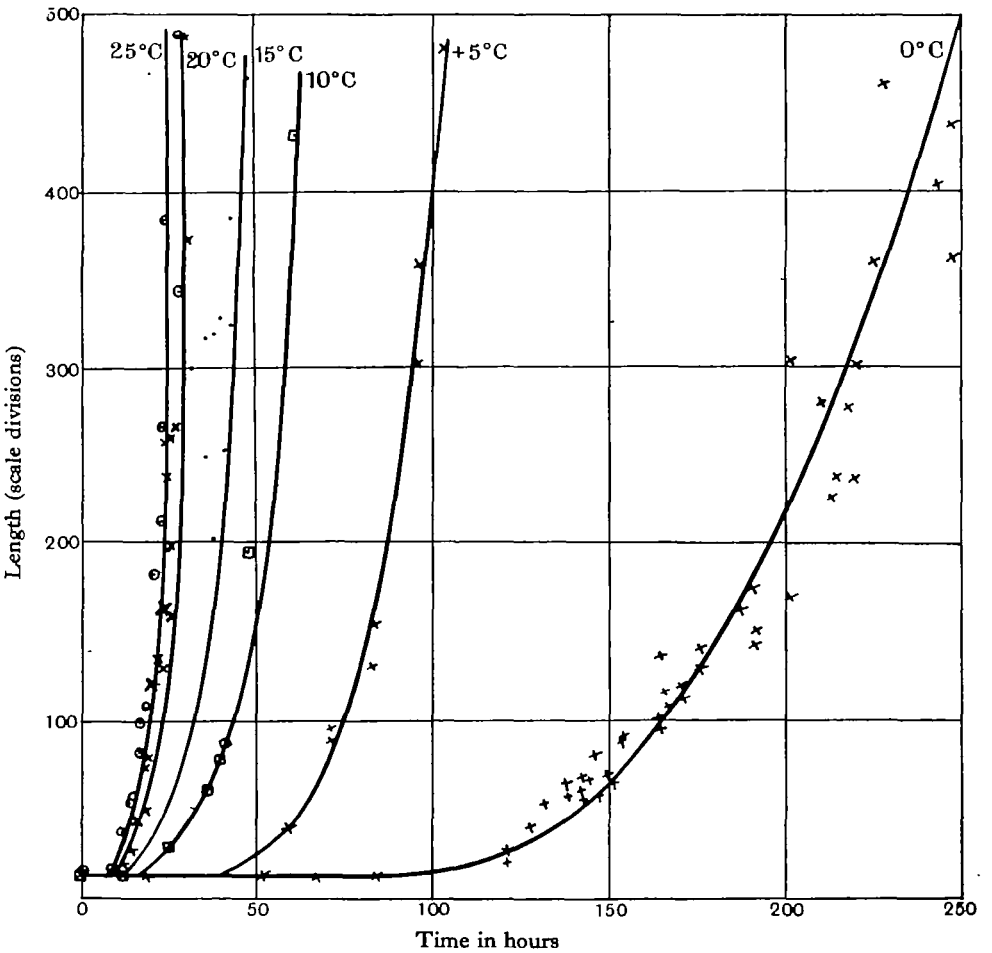

Fig. 2.

occurring. Provided that the measurements are confined to this earlier period, the error introduced in regarding increased length as an index of growth at − 5° C. is small.

For temperatures above zero about half a dozen preparations were made for each experiment and incubated together. Measurements were then made at random from one of the slides, followed by at least two others. In this way errors of sampling were reduced to a minimum. Although only a fraction of the total spores on

any one slide was measured at any one time, that fraction was composed of different individuals at each observation. By using a larger number of slides than was necessary, repeated measurements on one slide were avoided. In all cases at least two separate batches of slides have been used in order to show that the results obtained were repeatable. With those to be incubated below zero it was necessary to make more slides and to select those which had not frozen. At − 5° C. it was comparatively easy to supercool the agar, about 50 per cent. of the slides put down not crystallising. At − 7° C., however, only two of all the slides prepared did not crystallise initially, and removal of these to the microscope stage for measurement caused their subsequent crystallisation.

Growth was measured by means of a Ramsden micrometer eye-piece and a ⅛ in. oil-immersion objective. Over early periods of incubation the resulting threads were fairly straight and measurement was not difficult. After longer times the several bends and branches in the threads were measured separately as accurately as possible, and totalled. In all cases thirty measurements were made at random and each point in the curve is the mean of thirty such measurements, except in a few of the last measurements taken at the higher temperatures. There the threads were so intricately branched that measurement became difficult and laborious, and the mean of ten lengths had to be taken as representative. Reference to the logarithmic growth lines (Fig. 3) will show in general a falling off at the higher points. At the extremes of temperature—markedly above 25° C. and to some extent at − 5° C.—there seems to be definite departure from normal logarithmic growth during the periods of the experiments. At the other temperatures, this falling off must be ascribed to the difficulty of measurement of the longer threads, such lengths being under-estimates.

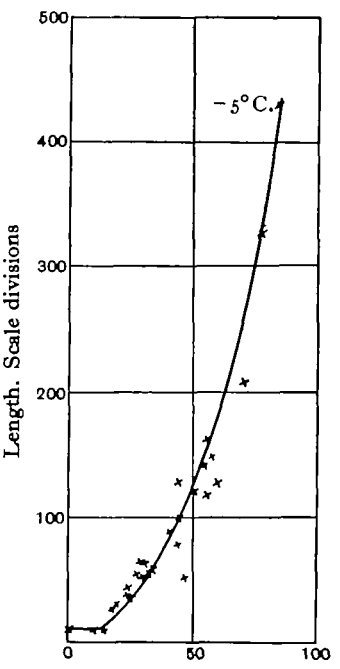

Fig. 2 A.

The mean results obtained are tabulated in Table I and shown graphically in Figs. 2 and 2 A. They are expressed as divisions of the micrometer scale. Calibration showed that one division of the Ramsden scale was approximately equivalent to 0·3μ. In Table II is drawn up a representative set of values obtained in one experiment at 20° C., in order to show the individual variation which occurred from thread to thread in a given selected group. It will be seen (Figs. 2 and 2 A) that there is an initial lag phase, followed by a period of logarithmic growth, that is, a period of rapid growth during which the logarithms of the length plotted against time yield a straight line. For present purposes there are included in the term "lag phase" both the "initial stationary phase" and the "lag phase" of Buchanan's nomenclature (Buchanan, 1918). If the length of "lag phase"

Table I. *Mean lengths of threads, in divisions of eye-piece scale. To convert to millimetres, one division of eye-piece is approximately equivalent to* 0·3 μ.

(*Time in hours.*)

25° C.		20° C.		15° C.		10° C.		+ 5° C.		0° C.		0° C.		− 5° C.		− 7° C.	
Hrs.	L.	Hrs.	L.	Hrs.	L.	Hrs.	L.	Hrs.	L.	Hrs.	L.	Hrs.	L.	Hrs.	L.	Hrs.	L.
0	13	0	14	0	12	0	14	0	12	0	12	190·5	144	0	12	0	13
8·5	15	11	17	13	15	12·5	13	52	12	18	12	192	163	336	12	1768	74
8·5	16	12	19	20	34	24·75	29	59·5	39	42·5	12	195·5	227	400	27	1768	69
11·75	37	14·25	27	21	37	35·75	61	71	96	67	12	199·5	199	425	30	—	—
14	53	16	44	25	42	40	69	71·5	89	84	12	201	203	521	44	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	552	37	—	—
15	55	16·5	50	35·5	142	40·5	87	83	131	120·5	20	201	170	571	36	—	—
16·5	99	17·75	83	36	316	47·75	196	83·3	155	121	27	209·5	281	639	65	—	—
—	—	—	—	—	—	—	—	—	—	—	—	—	—	648	54	—	—
17	81	18	74	36	249	61	433	95·5	303	128·5	42	212·5	227	664	64	—	—
18·5	107	19	80	38	318	62·25	543	96	360	131	54	213	227	672	52	—	—
20	110	20·25	120	38	202	67	625	103	482	137·5	65	214	239	768	56	—	—
21	183	21	134	40	328	67·75	553	119·5	684	139	59	218	278	792	58	—	—
22·5	212	22	163	40·5	253	—	—	120	592	142	60	220	302	936	89	—	—
23	266	22·5	164	42·5	386	—	—	121·5	691	142·5	68	220	238	1032	79	—	—
23·5	195	23	130	42·75	323	—	—	131·5	1056	143·5	59	225	362	1056	100	—	—
24	385	23·5	163	47	474	—	—	131·5	932	144	67	233	462	1106	51	—	—
27	456	24	257	47·25	464	—	—	—	—	146	82	243	403	1200	121	—	—
29	490	24·5	238	47·5	540	—	—	—	—	147	58	246·5	440	1296	141	—	—
29·5	344	25	258	—	—	—	—	—	—	150·5	70	247	363	1315	119	—	—
35·5	780	25	200	—	—	—	—	—	—	151	67	262	579	1323	162	—	—
—	—	26	159	—	—	—	—	—	—	154	92	284·5	622	1336	128	—	—
—	—	26·5	266	—	—	—	—	—	—	154	92	369·75	1351	1368	149	—	—
—	—	29	489	—	—	—	—	—	—	164·5	137	—	—	1674	208	—	—
—	—	30	373	—	—	—	—	—	—	164·5	103	—	—	1843	328		
—	—	30·5	608	—	—	—	—	—	—	165·5	97	—	—	2006	429	—	—
—	—	31	370	—	—	—	—	—	—	166·5	109	—	—	—	—	—	—
—	—	35·25	662	—	—	—	—	—	—	171	120	—	—	—	—	—	—
—	—	36	574	—	—	—	—	—	—	171	116	—	—	—	—	—	—
—	—	36·25	941	—	—	—	—	—	—	176	130	—	—	—	—	—	—
—	—	36·5	653	—	—	—	—	—	—	176	142	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	188	163	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	190·5	177	—	—	—	—	—	—

L. = Length

be plotted against temperature, a smooth curve results, showing that there is a definite though not a simple relationship between temperature and length of lag. Fig. 3 shows the logarithms of the lengths during the logarithmic growth phase plotted

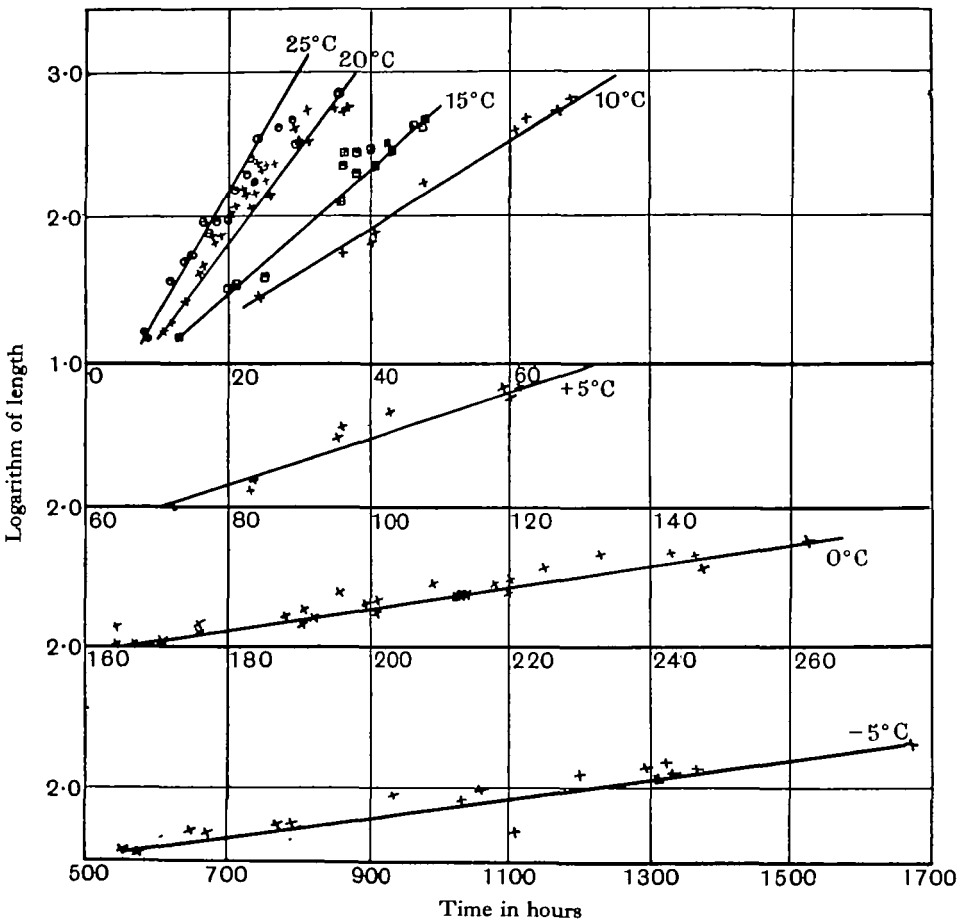

Fig. 3.

against time. The graph indicates that the gradients of the straight lines so formed decrease at first slowly, then more rapidly. Using the expression

$$\tan\theta = \frac{\log L_2 - \log L_1}{T_2 - T_1},$$

where θ = gradient, L_2 = length at time T_2, L_1 = length at time T_1, the mean value of the angle of slope of each line for any given temperature can be calculated, in terms of the arbitrary units of time and length used. Omitting values departing from the mean value, and multiplying by ten for convenience, the mean values so obtained are given in Table III and graphically in Fig. 5. The points lie in a smooth

curve approaching the temperature axis asymptotically, but becoming very close to this axis at a temperature of − 10° C. In other words growth on supercooled Czapek's agar becomes infinitely slow at − 10° C., which is in accord with the very slow rate of growth observed experimentally on the two slides which it was possible to supercool to − 7° C.

Table II. *A representative set of values obtained in one experiment at* 20° C.

Scale divisions						
Time in hours						
0	12	14·25	17·75	21	25	36·25
14	18	35	27	170	234	933
15	20	34	28	64	335	938
15	27	19	49	91	87	831
12	31	18	44	195	178	695
14	14	18	18	74	183	880
14	13	20	161	63	360	1189
11	16	20	57	58	114	1151
10	25	36	34	185	335	1361
12	15	18	62	207	352	979
	15	19	40	138	190	1059
	18	59	88	63	153	1129
	15	22	100	56	313	873
	13	35	69	95	274	1091
	11	27	85	32	514	619
	18	14	61	69	145	860
	15	53	62	334	159	955
	13	16	84	247	194	941
	17	25	141	76	506	1210
	13	27	90	183	533	1350
	38	33	94	62	379	909
	19	22	56	130	253	571
	19	23	49	141	186	918
	35	20	98	79	195	813
	19	22	67	40	210	762
	14	52	34	75	111	584
	13	38	179	103	218	777
	15	20	100	197	403	520
	17	32	222	308	284	646
	20	20	210	180	193	1677
	15	16	78	302	155	1207
Mean 14	19	27	83	134	258	941

Table III. $\tan\theta = \dfrac{\log L_2 - \log L_1}{T_2 - T_1}$.

The value of tan θ was calculated for each point on the straight line of logarithmic growth, and the mean value of tan $\theta \times 10$ is given below.

Temperature	tan $\theta \times 10$
25° C.	0·87
20° C.	0·81
15° C.	0·48
10° C.	0·32
+ 5° C.	0·18
0° C.	0·07 (5)
− 5° C.	0·01 (2)

APPLICATION OF THE ARRHENIUS EQUATION.

The expression used above for the calculation of tan θ is in effect a measure of the velocity constant of growth (k). Thus from the equation

$$\frac{dL}{dt} = kL,$$

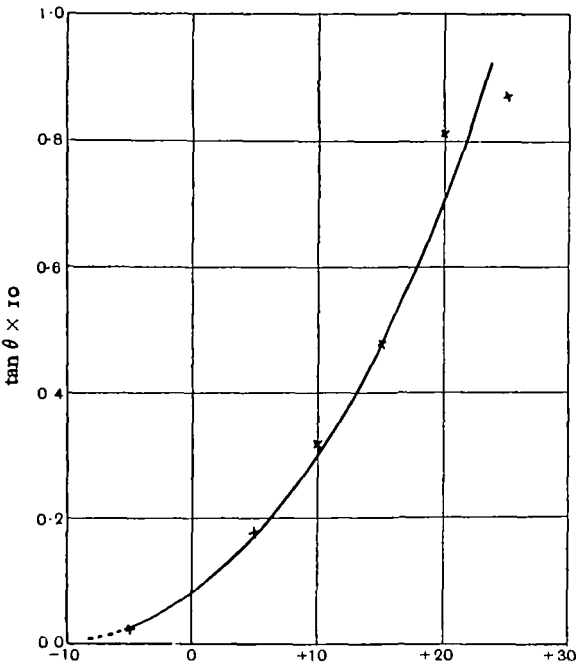

Fig. 4.

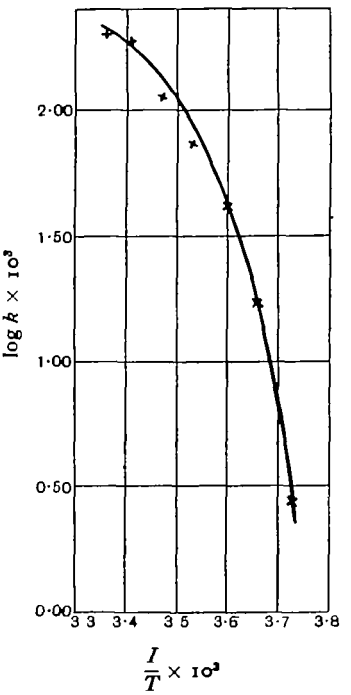

Fig. 5.

where L = length of thread, t = time, for the rate of growth during the logarithmic phase, it follows that

$$k = \frac{\log_{10} L_2 - \log_{10} L_1}{T_2 - T_1} \times 2{\cdot}303,$$

where L_1 = length of thread at time T_1, L_2 length of thread at time T_2, that is,

$$k = \tan\theta \times 2{\cdot}303.$$

The form of the Arrhenius-van't Hoff equation generally used in biological work

$$\frac{d \log_e k}{dT} = \frac{-\mu}{RT^2} \qquad \ldots\ldots(1),$$

μ = "critical thermal increment," indicates that log k plotted against the reciprocal of absolute temperature should yield a straight line. Values of k obtained from the experiments with *Sporotrichum* are tabulated in Table IV, and are shown graphically

plotted against $\frac{I}{T}$ in Fig. 5. It will be observed that they lie not on a straight line but on a continuous curve.

Table IV.

Temp. °C.	$\frac{I}{T}$ absolute $\times 10^3$	log k from experimental results $\times 10^3$	log k from smoothed curve $\times 10^3$	μ calculated from experimental values of k	μ calculated from smoothed values of k from Fig. 5
25	3·36	2·30	2·30	2,495	4,830
20	3·41	2·27	2·24	17,670	10,110
15	3·47	2·04	2·11	13,290	14,280
10	3·53	1·87	1·92	18,050	23,930
+ 5	3·60	1·62	1·59	26,640	30,090
0	3·66	1·24	1·46	53,570	48,560
− 5	3·73	0·44	0·44	—	—

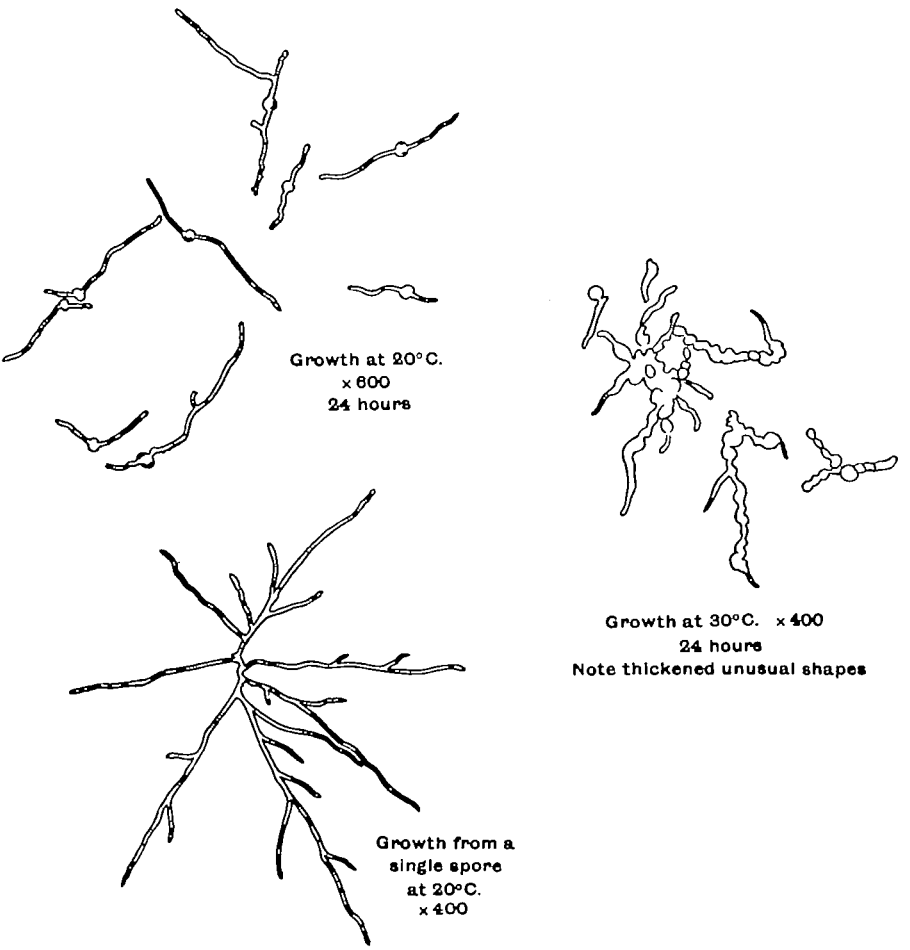

Fig. 6.

Integration of equation (1) gives

$$\mu = \frac{4{\cdot}606\ (\log k_2 - \log k_1)\ T_1 T_2}{T_2 - T_1},$$

T_1 and T_2 being absolute temperatures. Values of μ can be calculated from this equation. These also are given in Table IV. Here again μ is not constant but varies continuously with the temperature: a finding in accord with the views of Fulmer and Buchanan as opposed to those of Crozier (Fulmer and Buchanan, 1929, and Crozier, 1926).

SUMMARY.

Measurements of the rate of growth of *Sporotrichum carnis* on Czapek's agar indicate that:

1. The optimum temperature for growth is at 25° C.
2. Growth can take place at 30° C., but is restricted and unusual shapes are produced by the thickening of the germ tubes.
3. Fairly good, though slow, growth was obtained at − 5° C. on supercooled agar, and growth also took place at − 7° C. on supercooled agar. There are indications, however, that growth is somewhat restrained at − 5° C.; the germ-tubes become thicker and curl more readily than at higher temperatures.
4. In no case was growth observed on *frozen* agar during the periods of incubation of the slides—up to two months.
5. A curve is given showing the relation between temperature and rate of growth during the logarithmic phase. The form of the curve suggests that growth on supercooled agar becomes infinitely slow at − 10° C.
6. Application of the Arrhenius-van't Hoff equation to the results obtained is considered.

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