PRELIMINARY EXPERIMENTS ON THE INFLUENCE OF NEUTRAL SALTS ON THE GERMINATION OF LATHYRUS-POLLEN

by

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1. Introduction.

The experiments described below were already performed in 1928, but because of the fact that we do not intend to continue this work, we publish the results together with a program of a possible future systematic extension, in order that the material might be compared with the behaviour of colloidal models (mainly autocomplex-coacervates of phosphatids) [1]) which have been studied by us in the past few years.

2. Material and Methods.

The pollen from a single flowering branch of Lathyrus odoratus L. (garden-variety) is shaken from the flowers and collected on a watchglass. A small amount (more than 500 grains) are brought in 6 c.c. of fluid in a watchglass. (diameter 10 cm.). The grains explode in distilled water. Germination (21° C) appeared to be optimal in 15 % saccharosesolution (as compared with 0, 5, 10, 15, 20, 25 and 30 $^0/_0$). As standard solution we used, therefore, 3 cc. 30 % saccharosesolution + 3 cc. neutral-salt-

[1]) H. G. Bungenberg de Jong and R. F. Westerkamp: Bioch. Zs. Bd. 248, pg. 131, 309 and 335, 1932.

solution). The percentage of germination was determined after 4 hours, the visibility of the pollen-tube being selected as a criterion of germination (regardless of the length of the pollen-tube). Even if the pollen-tube is barely visible as a small spot, the grain is considered to germinate. On four to eight different places on the watchglass the number of pollen grains visible in the field was counted, and the number of non-germinating grains (or, in the case of a low percentage of germination, the number of germinating grains) was counted. The numbers in the following tables each represent 250—800 pollen-grains counted. Often the pollen is crowded along the rim of the glass, at the margin of the fluid-meniscus. This behaviour does not seem to influence the percentage germination.

3. Influence of neutral salts on the percentage of germination.

Table I and figure 1 refer to an experiment on pollen mixed from three flowers on the same branch. In three

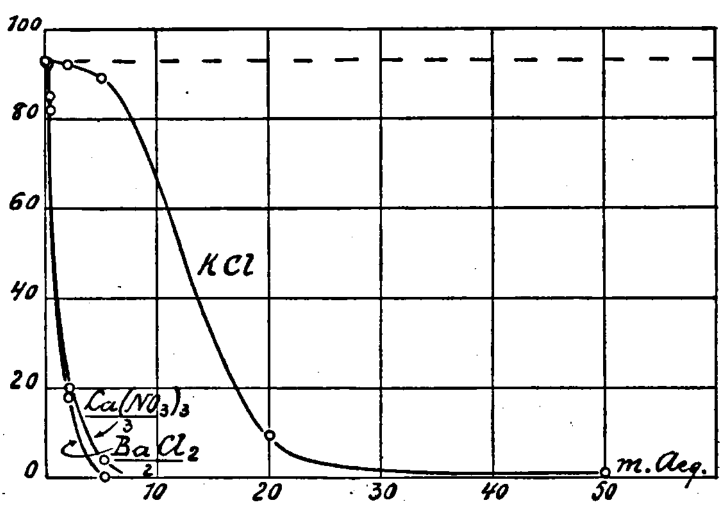

Fig. 1.

blank experiments the pollen of each flower was tested individually, thus showing the material to be homogeneous (table I). Figure 1 (ordinates: % germination, abcissae: concentration of salt in milli-aequivalents) shows that the intensity of germination is depressed by minimal concentrations of neutral salts. An osmotic effect, therefore, may hardly account for this phenomenon. The pollen is, moreover, rather insensitive to changes in the osmotic pressure of the milieu, as germination in 20 % saccharose was depressed but little.

TABLE I.

Milieu		Percentage-germination	Length of the pollen-tube
Blank		93, 92, 94 } 93	267, 211, 240 } 239 units
KCl	0.5 m.aeq.	92	260
	2 "	92	209
	5 "	89	204
	20 "	9	63
	50 "	1	15
$BaCl_2$	0.5 m.aeq.	82	185
	2 "	18	84
	5 "	0	—
	20 "	0	—
	50 "	0	—
$La(NO_3)_3$	0.5 m.aeq.	85	249
	2 "	20	159
	5 "	4	95
	20 "	0	—
	50 "	0	—

4. The affinity of the curves obtained.

Figure 2 shows the same data as figure 1, the concentrations of the neutral salts, however, are plotted logarithmically. The affinity of the curves, and therefore the

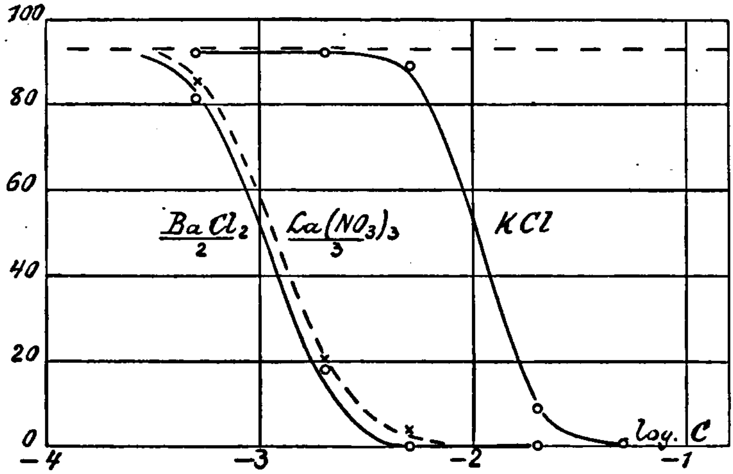

Fig. 2.

affinity of the various salt effects, seems evident. The advantages of such a logarithmic representation for similar researches are:

1. The salts which are active in low concentration are, graphically „at a disadvantage" when we plot arithmetically. They have „equal rights" when plotted logarithmically.
2. It may appear that the logarithmic x-distance, representing the transition from the level of the „blank" (broken line) to the abcissae, happens to be the same for various salts.
3. If the S-shaped curves belong to the same family, the transition-range-may be characterised by but one parameter, which is log (concentration) of a comparable point on each of the curves.

If the curves do not belong to the same family, the point of inflexion or, (in case the curve shows no symmetry) one half of the maximal ordinate may be used as a preliminary characteristic.

5. The peculiar behaviour of Ca-ion.

In the following series the pollen from another flowering branch was used to ascertain the influence of NaCl, KCl

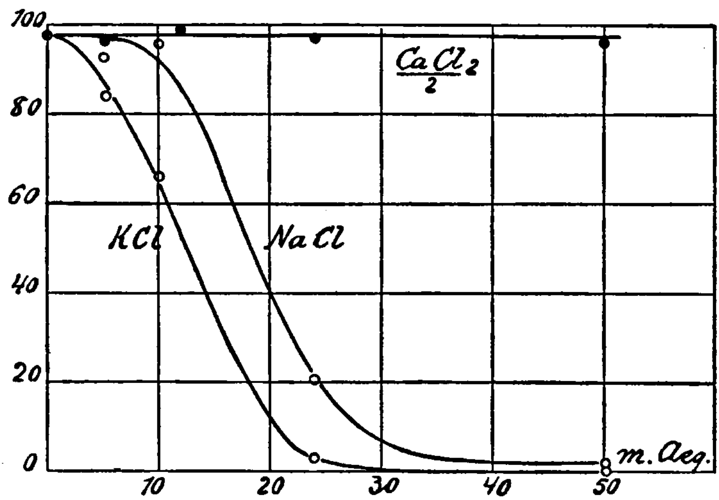

Fig. 3.

and $CaCl_2$ (Table II and figure 3). It may be seen that both NaCl and KCl depress the germination, while $CaCl_2$, even in the highest concentrations investigated, appears to have no effect whatsoever. The difference of Ca^{++} and Ba^{++} (figure 1) is apparent. Further experiments, at higher Ca^{++}-concentration have to show whether this ion yields a similar type of curves as obtained for the other ions. From the point of view of the colloidchemist the relative sequence of the affine curves for the chlorides of Li, NH_4, Na, K, Rb, Cs and also of Mg, Ca, Sr and Ba has to be investigated.

6. Antagonism Na-Ca and K-Ca.

With the same material used for experiment 5 we tested the influence of salt-mixtures. In these mixtures the NaCl, resp. KCl concentration was kept at 25 milli-aequivalents, while the $CaCl_2$ concentration varied. It appears that already 2.5 m.aeq. $CaCl_2$ effectively detoxifies the solution of NaCl or KCl. It seems desirable to continue these experiments with lower concentrations of $CaCl_2$. In a graphical representation we might expect (figure 2) „inverted" S-shaped curves, which mode of representation posesses the advantages already mentioned under 4.

TABLE II.

Milieu	Percentage Germination
Blank	98
KCl 5 m.aeq.	84 (too low?)
10 „	66
25 „	3
50 „	0
NaCl 5 m.aeq.	93
10 „	96
25 „	21
50 „	2
$CaCl_2$ 5 m.aeq.	96
10 „	99
25 „	97
50 „	96
KCl 25 m.aeq. $CaCl_2$ 2.5 m.aeq.	92
„ 25 „ „ 5 „	97
„ 25 „ „ 12.5 „	95
„ 25 „ „ 25 „	96
NaCl 25 m.aeq. $CaCl_2$ 2.5 m.aeq.	96
„ 25 „ „ 12.5 „	96

7. **The unsuitable nature of agar-plates,** as often used in a medium, appears from the following considerations. Agar contains Ca- (and other) ions which are already

liberated (as shown by qualitative test with NH_4-oxalate) on contact with distilled water. Washed agar still contains Ca-ions, which are readily exchanged when the agar comes in contact with a salt-solution with other kations.

8. The growth-rate of the tube is not well suited as a criterion of salt-effects.

In the experimental series, plotted in Table I (figure 1) the tube-length of 15 grains was determined. The average, expressed in arbitrary units, is shown in column 3, Table I. Figure 4 depicts the results. It may be observed that the

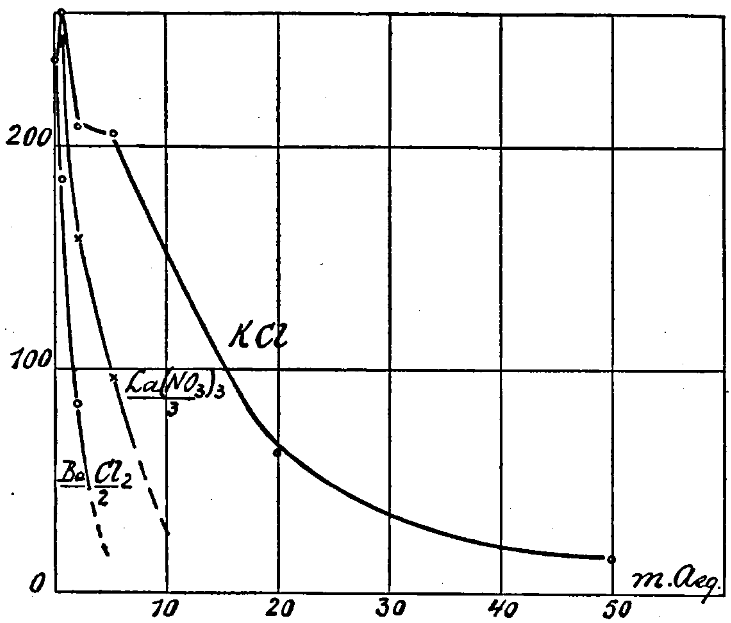

Fig. 4.

effect is similar as that obtained by counting the pollen-grains, but the statistical average is, of course, less reliable for *measuring*, as it is for *counting* („plus or minus" criterion). The time needed for measurement of a sufficient number of pollen-tubes showed to be disproportionally long as compared to the results obtained by counting (compare the discontinuities in the KCl-curve, figure 4 with the continuous KCl-curve in figure 1).

For the sake of completeness we mention the fact that the average tube-length in solutions of $CaCl_2$ appears to be greater than in the control-solutions.

9. For a possible continuation of these experiments the following points seem to us to be desirable.

In order that the results of a pollen-investigation may yield material which is comparable with the colloid-experiments mentioned above, the influence of kations and anions of higher valence should be investigated. Not all polyvalent ions are suitable for this purpose. Aluminum- and Thorium-salts should be avoided (danger of undesirable pH-influence and complications, resulting from the formation of hydroxyde). Even the Lanthanum-nitrate, used in our experiments, is far from ideal. The so-called complex-chemistry yields a few suitable salts with polyvalent kations (3—4—6-valent): $Co(NH_3)_6Cl_3$ = luteo-cobaltchloride, $\left[Pt(en)_3\right](NO_3)_4$ and $\left[Co\left\{Co(en)_2(OH)_2\right\}_3\right](NO_3)_6$ hexol-nitrate.

For experiments on biological material these salts have been succesfully used in our laboratory. Of the polyvalent anions $Fe(CN)_6^{\equiv}$ is suspicious (the solutions are decomposed by the light and become alkaline), the use of $Fe(CN)_6^{\equiv}$ is perhaps admissible. An eminently useful salt with a trivalent anion is potassium-methane-trisulfonate: $CH(KSO_3)_3$.