BALDWIN

The Effect of Alcohol and Chloroform on the Grains of Indian Corn

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THE EFFECT OF ALCOHOL AND CHLOROFORM ON THE GRAINS OF INDIAN CORN

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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The Effect of Alcohol and Chloroform on the Grains of Indian Corn.

The length of time during which seeds under various conditions retain their vitality is a subject which has interested numerous investigators. Seeds of various kinds have been subjected to extremes of temperature, to the prolonged action of certain gases, and to treatment with different poisonous liquids, with the result that dry seeds seem very resistant to conditions which do not favor growth. Such experiments are of interest because of their value in the investigation of the nature of living matter. From well directed experiments it has been concluded that living matter may exist without any chemical change, and may therefore, retain its vitality in a condition of suspended animation for an indefinite period of time. Other experiments dealing with the influence of poisons upon plant organs have suggested that the marked resistance of many seeds to certain poisons is due, not to the stability of quiescent protoplasm, but to the imperviousness of the seed coat. The seed coats of various grasses have been found to behave as a semi-permeable membrane, allowing certain substances to pass through but preventing the entrance of others. In a number of cases it has been well established that this membrane when dry is quite impervious to certain anhydrous liquids and gases. On the other hand, when the membrane contains definite amounts of moisture it becomes readily permeable to these agents. The same is true when water is added to the agent itself.

In the literature upon the subject, more particularly in that relating to the effect of alcohol and chloroform, no mention is

found concerning the effect of these agents upon the grains of Indian corn. The present series of experiments was undertaken for the purpose of ascertaining the effects of chloroform and of a wide range of dilutions of alcohol upon the germination and growth of Indian corn,and further of comparing its behavior with that of seeds used in other experiments.

II. Method.

Care was taken in the first place to secure good seed corn for these experiments,none being used which would not under favorable conditions germinate very close to 100 per cent. Different varieties were used,namely,Abbott's White,Iowa Silver Mine,Reid's Yellow Dent,Funk's Golden Dent,and a variety of high oil corn bred by the Illinois Agricultural Experiment Station. This latter averaged 7 % in oil content,3 % more than is commonly found in corn. Parallel experiments showed no appreciable difference in the behavior of these varieties,with the exception of the corn of high oil content. In the experiments that follow the variety used is noted.

Corn immersed in chloroform and in ethyl alcohol was kept in blue bottles with ground glass stoppers. The stoppers were coated with vaseline and the bottles kept in the dark. Corn was immersed in alcohol of the following per cents - 10,20, 30,40,50,60,70,80,90,95,and absolute. The grades from 10 to 90 % inclusive were made in quantities by means of Tralle's alkoholometer and kept in carefully stoppered bottles. Tests made from time to time of the stock solutions showed little,if

any, change. The absolute alcohol was tested for freedom from water by means of $CuSO_4$. The chloroform was tested for phosgene and HCl both before and after using by means of the HNO_3 and $AgNO_3$ test. It was always found to be free from these poisons.

Quantities of corn were treated with chloroform and with the grades of alcohol for varying periods of time and under different conditions. The effect in each case was determined by the number of grains germinating. For germination the grains were placed between filter paper this between damp towels in fiber tubs or granite iron pans. The moisture content was maintained as uniformly as possible and never allowed to range too high. The germinators were kept, for the most part, in a laboratory the temperature of which varied between 18° and 26° C. As is apparent from the tables, the temperature variation within the limits indicated, did not materially affect the results.

The greatest difficulty was experienced with moulds, mucor, aspergillus, and penecillium finding conditions favorable for rapid growth. Though care was used in the selection of the corn many grains were found to have developed mycelium and fruit within the seed coats. Various methods of combating moulds were tried, but the one finally resorted to was that of wiping the mycelium from the outside of each grain at least once a day until germination was well started. By doing this the mould could be held in check.

The standard taken for germination was that stage where there was distinct growth of radicle, coleoptile, or in some cases the development of secondary roots. This may give figures for

germination which are a little high,since many of the seedlings were so weak or so poorly developed that they would not have reached maturity.

III. Experiments.

Part I. To determine the effect of different grades of ethyl alcohol on air dried grains of corn.

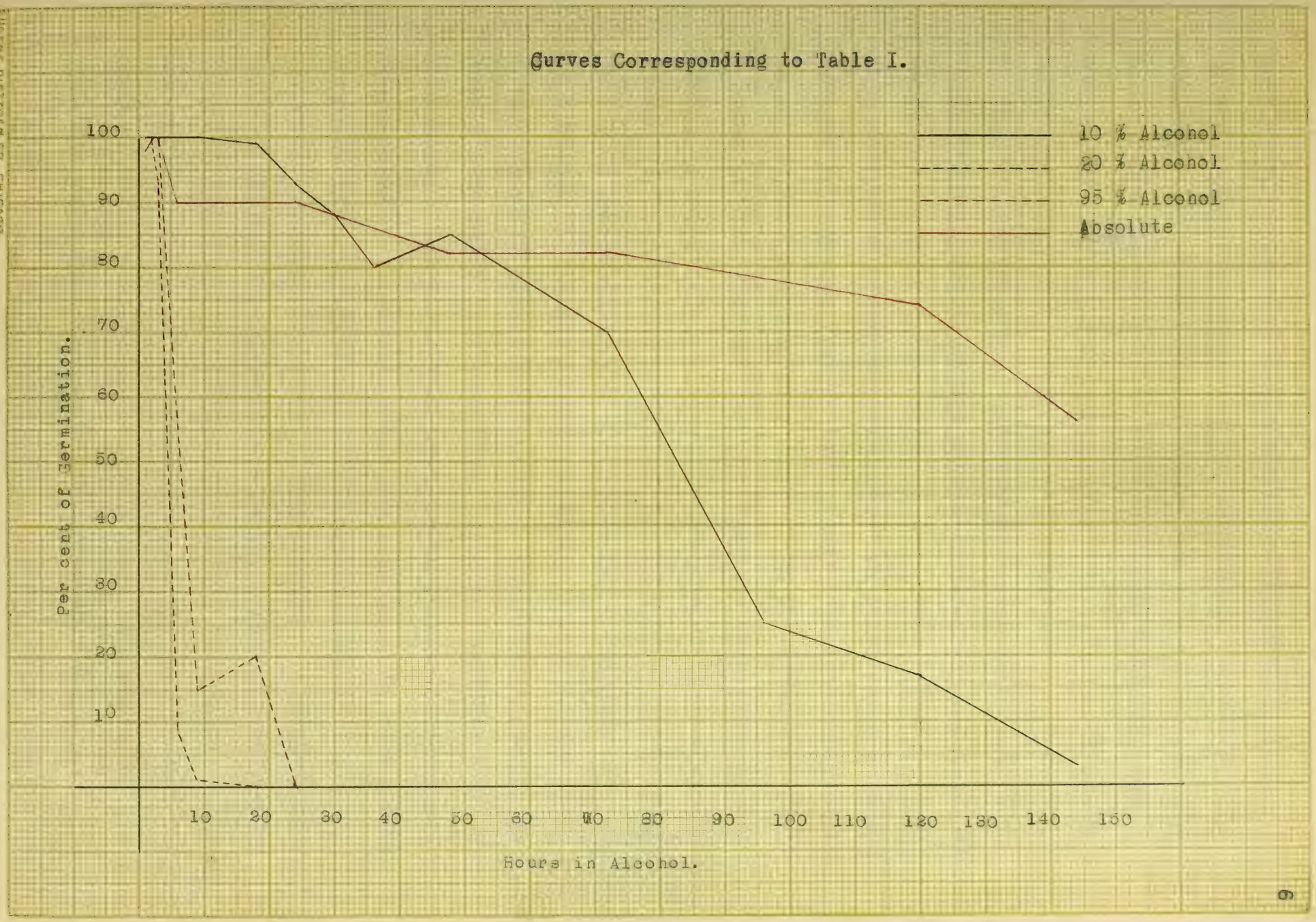
Preliminary experiments showed a marked difference in the effect of different grades of alcohol upon the germination of grains and the growth of seedlings,and for this reason the following series of experiments was carried on. The corn used was chiefly Reid's Yellow Dent,though some experiments were made with Iowa Silver Mine and Funk's Golden Dent and no difference in behavior was noted. Air dried grains were immersed in the various grades of alcohol for different periods of time and then placed directly into germinators as already described. The following table gives results of this series as shown by the germination test.

As shown by this table 70 and 80 % alcohols were most injurious and 10 % and absolute least so,as judged by the per cent of germination. The deleterious effect of alcohol was also shown in the injured or poorly developed seedlings and in a surprisingly short time. Where grains were subjected for 1 hour to the action of 60,70,and 80 % the radicles showed serious injury,growing very little or not at all. In the former case the coleorhiza showed very little growth. (See drawings on page 42) Secondary roots developed early and became prominent in these seedlings.

Table I.

Corn in grades of alcohol for different periods of time.

Hours	1	2	3	6	9	18	24	30	36	48	72	96	120	144
H_2O	100	100	100	100	100	99	100					100		1
10 %	100	100	100	100	100	99	93	88	80	85	70	25	17	3
20	100	100	93	9	1	0	0							
30	100	92	50	0	0	0	0							
40	97	65	30	0	0	0	0							
50	93	48	8	0	0	0	0							
60	82	33	0	0	0	0	0							
70	15	0	0	0	0	0	0							
80	19	0	0	0	0	0	0							
90	98	60	28	0	0	0	0							
95	100	100	100	57	15	20	0							
Absolute	98	100	100	90			90			82	82		74	56


Curves Corresponding to Table I.
10 % Alcohol
20 % Alcohol
95 % Alcohol
Absolute
Per cent of Germination.
100
90
80
70
60
50
40
30
20
10
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
Hours in Alcohol.

Many grains which were treated with 50 and 60 % for 1 hour showed considerable growth of coleoptile and secondary roots under the coat of the kernel before any part of the seedling could force its way out. Similar effects were seen when grains were treated for longer periods of time, becoming more marked as the time of immersion was lengthened. For example, in the set treated for 3 hours the seedlings from 30 to 90 % inclusive were feeble, having radicles poorly developed and with seldom any growth of coleorhiza, or more often killed. In this set growth under the seed coats was conspicuous in those treated with grades from 20 to 90 % and occurred occassionally where 95 % and absolute were used. 20 % alcohol acting for 6 hours produce distinct injury. Coleorhizas often showed little if any growth and some radicles failed to develop. A period of 18 hours was sufficient time for 95 % to injure the embryo, as was shown by the weak seedlings developing from grains so treated. Alcohol of 10 % produced similar injury to a large number of grains in 24 hours. Radicles in many cases were killed, while in others there was poor development of this structure. In the latter case the coleorhiza often showed normal growth. Injured seedlings from this lot were planted in earth with the result that they grew, but did so more slowly than did uninjured seedlings of the same age. Grains immersed in 10 % alcohol for 30 hours and longer produced seedlings in which, in the majority of cases, the radicle was killed. Where the radicle did grow the coleorhiza usually grew also. In many of these seedlings there was a great enlargement of the caulicle accompanied by poor development of radicle and

coleoptile. For illustrations of these various points see drawings pages 41-42.

The injurious effect of alcohol upon corn embryos was also shown in the delayed germination of grains treated with this agent. This is shown in tables III A, IV A, and V A which give the germination by days of grains treated with grades of alcohol for 1,2, and 3 hour periods. Those grades of alcohol which most seriously affected germination and development, also caused the greatest delay in germination. That delayed germination is one of the first effects of alcohol, is seen from table II, which gives the germination by days when grains were treated with 60, 70, and 80 % alcohol for 20 and for 40 minutes. These short periods of time were not sufficient to cause great decrease in per cent of germination, nor to bring about marked injury to embryos, though some grains kept in these grades for 40 minutes showed poorly developed radicles. Corn used was Reid's Yellow Dent and 20 grains were used for each test. This experiment was started March 1.

Table II.

Days	1	2	3	4	5	6	7	8	Total No.	% of Germination
20 min. in H_2O				3	9	8			20	100
" 60 %				2	4	12	1		19	95
" 70 %				0	4	9	1	1	15	75
" 80 %				1	0	12	7		20	100
40 " in H_2O				4	8	8			20	100
" 60 %				4	10	4			18	90
" 70 %					8	5	2		15	75
" 80 %				1	9	6	1		17	85

Not only is delay in germination noted as a result of treatment with alcohol,but the rate of growth is much retarded as compared with normal seedlings of the same age. Grains subjected to the different grades of alcohol for 3 hours showed this variation in the rate of growth. Grains treated with 10 % alcohol for this time showed very slight retardation when compared with the check,whereas those germinating on the same day from the lot treated with 20 % showed this distinctly. Such retardation appeared greater with each succeeding per cent up to 50 %. Where 60,70,and 80 % were used there was no germination of grains. With 90 % the rate of growth increased, and with 95 % and absolute was about equal to the check.

Reference has been made to the fact that some grains showed considerable growth of coleoptile and secondary roots underneath the coat. In order to determine whether the embryo is so weakened by the action of alcohol that the seedling is often unable to force its way through the covering of the grain, or whether this covering is made more difficult to break because of the treatment,a set of experiments was performed as follows. Intact grains of Iowa Silver Mine corn were subjected to the various grades of alcohol for 1,2,and 3 hours,80 grains being used in each test. Upon removal from the alcohol the seed coats were stripped from the embryo sides of half the grains of each lot,and these together with those whose coats were not removed were placed in a large tray where all were under approximately the same conditions of moisture and temperature. Results are shown in the following tables. These experiments were started March 23.

Table III A. 1 hour in agent, coats not removed.

Days	23	24	25	26	27	28	29	30	31	1	2	3	Total	%
H_2O			8	16	15	1							40	100
10 %			10	9	21								40	100
20			4	18	18								40	100
30				25	15								40	100
40				19	17	0	2						38	95
50				7	22	1	6	1	0	1			38	95
60				1	8	3	6	5	7	2	4		36	90
70					1	1	0	2	0	2	2	1	9	22.5
80					1	5	2	2	0	1	1	3	15	37.5
90				15	23	1	0	1					40	100
95			2	21	16	1							40	100
Absolute			5	23	12								40	100

Table III B. 1 hour in agent, coats removed.

Days	23	24	25	26	27	28	29	30	31	1	2	3	Total	%
H_2O			9	19	12								40	100
10 %			10	20	10								40	100
20			5	19	16								40	100
30			2	24	14								40	100
40				14	26								40	100
50				15	25								40	100
60				11	26								37	92.5
70				8	22	4							34	85
80				4	24	6							34	85
90				26	14								40	100
95			4	25	11								40	100
Absolute			4	13	22	1							40	100

Table IV A. 2 hours in agent, coats not removed.

Days	23	24	25	26	27	28	29	30	31	1	2	3	Total	%
H_2O			10	16	14								40	100
10 %			8	17	15								40	100
20			4	17	19								40	100
30				4	31	1							36	90
40					10	5	5	0	0	0	1		21	50.2
50					3	6	1	2	2	0	3		17	42.5
60					1	0	1	0	0	2			4	10
70													0	0
80													0	0
90				3	29	0	1						33	82.5
95				7	33								40	100
Absolute				11	29								40	100

Table IV B. 2 hours in agent, coats removed.

Days	23	24	25	26	27	28	29	30	31	1	2	3	Total	%
H_2O			2	12	24	2							40	100
10 %			7	17	16								40	100
20			3	21	16								40	100
30				19	18	2							39	97.5
40				16	15								31	77.5
50				13	13	0	1						27	67.5
60				1	14								15	37.5
70					3	2	1						6	15
80					4	1							5	12.5
90				13	23	1	1						38	95
95				20	19								39	97.5
Absolute			3	16	20								39	97.5

Table V A. 3 hours in agent, coats not removed.

Days	23	24	25	26	27	28	29	30	31	1	2	3	Total	%
H_2O			1	c20	16	3							40	100
10 %				12	28								40	100
20				16	18	3	3						40	100
30				1	11	1	2	2	2	3	1		23	57.5
40					6	2	0	2	0	1			11	27.5
50					1	1							2	5
60													0	0
70													0	0
80													0	0
90					7	1	4	1	1	1			15	37.5
95					30	3	3	4					40	100
Absolute				4	28	3	2	3					40	100

Table V B. 3 hours in agent, coats removed.

Days	23	24	25	26	27	28	29	30	31	1	2	3	Total	%
H_2O			2	30	8								40	100
10 %			2	34	4								40	100
20			1	36	3								40	100
30				25	8								33	82.5
40				12	12								24	60
50				7	5								12	30
60					2								2	5
70													0	0
80													0	0
90				12	16	1							29	72.5
95				35	4								39	97.5
Absolute			3	32	5								40	100

From this data it is evident that the seed coat does not interfere with the development of the stronger seedlings, that is those which germinate first. But the removal of the coat seems to assist in the case of the weaker ones which, when the coats are left intact develop only after some delay or not at all. The standard set for germination was the first distinct growth in the plant axis, but many of the seedlings especially in lots III B, IV B, and V B while showing this initial growth, were weak and poorly developed. Grains treated in the manner just described were planted in earth with the result that practically no difference could be seen in the two lots (those left intact and those with the coats removed) in the number of grains germinating, and little difference in the time of germination. The retardation of growth mentioned before is seen in these seedlings, those treated with 10 % growing more rapidly than the grains treated with 20 %, and the latter showing an increase over those treated with 30 %. All the seedlings from grains treated with a given grade of alcohol for a given period of time, whether the grains were subsequently left intact or not, showed approximately the same vigor and rate of growth. The results of these experiments would indicate that the embryo is weakened by certain grades of alcohol and, while it may be able to begin growth, especially if the seed coat is removed, it does not have sufficient vitality to develop further and push its way th through the soil.

Part II. To determine the relation of the seed coats to the penetration of alcohol and chloroform and to the consequent injury of the embryo.

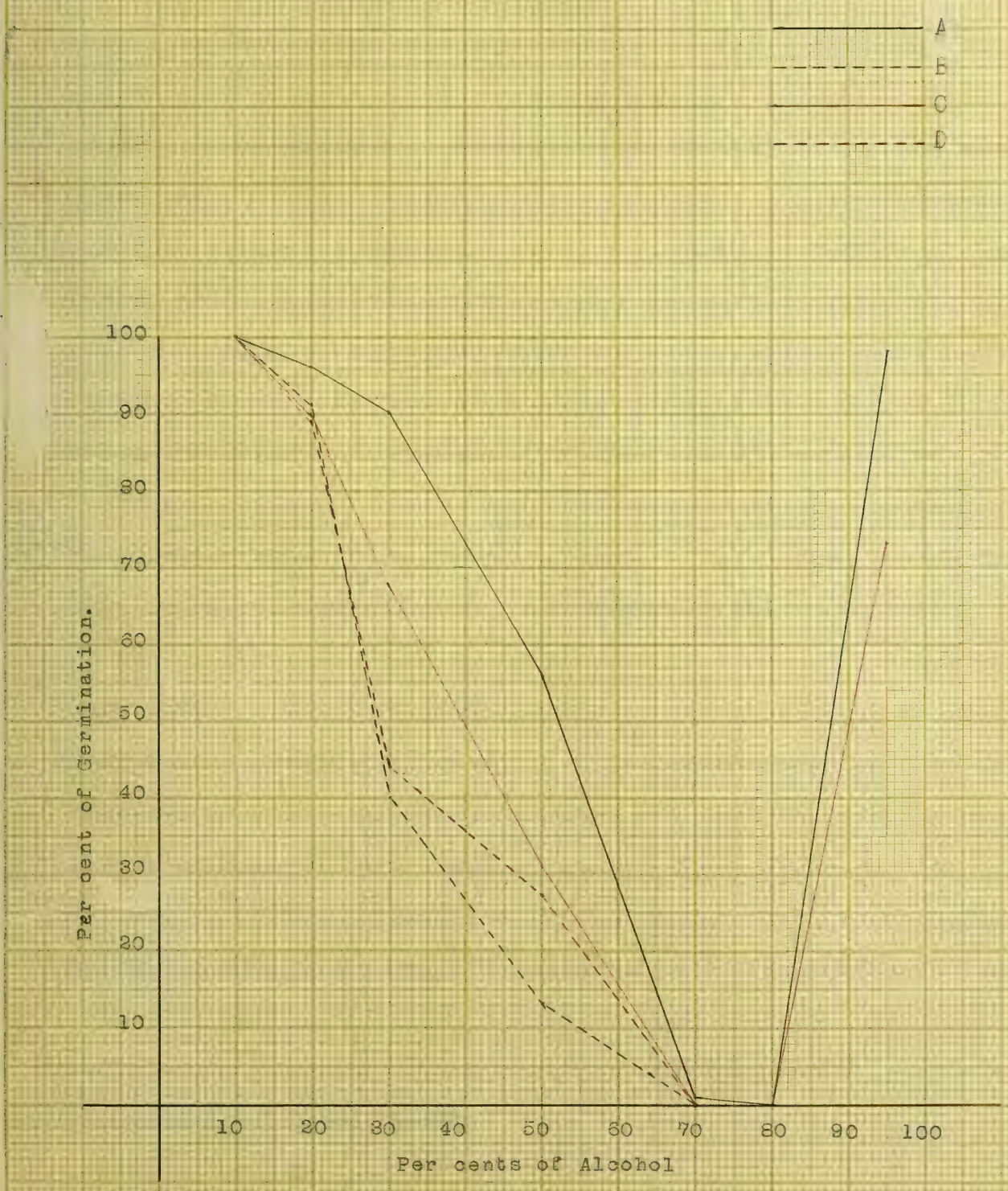
After various preliminary experiments, the results of which seemed to indicate that the coats of the corn kernel checked the entrance of the agent, experiments were carried on in the following manner. The corn used for these experiments was Reid's Yellow Dent, and 100 grains were used for each test. These grains were divided into four sets-- (A) with coats left intact, (B) coats cut away from the tip of the grain but in such a way as not to injure the coleorhiza, (C) coats and a portion of the endosperm cut from the top of the grain but leaving the coleoptile uninjured, (D) coats cut away from the entire embryo side of the grain. Grains so treated were placed in the various grades of alcohol, in chloroform, and in water. Those in chloroform and absolute alcohol were left for 4 hours while the remainder were left for 2 hours only. The grains were then put directly into pans arranged as germinators. The following table gives the results of the experiments as shown by the total number of grains germinated.

In rate of growth and development of the various parts these seedlings showed considerable variation. The grains treated with 10 % alcohol developed in every way as did those treated with water alone. Delay in germination and retardation in rate of growth similar to that noted in Part I was observed in this experiment. Among the grains treated with 20 % alcohol the ones stripped on the embryo side showed much injury to the radicle, this structure growing only little or not at all.

Table VI.

		No. Germinated			No. Germinated
H_2O	A	100	70 %	A	1
for 2 hours	B	100	for 2 hours	B	0
	C	100		C	0
	D	100		D	0
10 %	A	100	80 %	A	0
for 2 hours	B	100	for 2 hours	B	0
	C	100		C	0
	D	100		D	0
20 %	A	96	95 %	A	98
for 2 hours	B	91	for 2 hours	B	0
	C	90		C	73
	D	89		D	0
30 %	A	90	Absolute	A	93
for 2 hours	B	40	for 4 hours	B	0
	C	67		C	79
	D	44		D	0
50 %	A	56	Chloroform	A	99
for 2 hours	B	13	for 4 hours	B	92
	C	31		C	99
	D	27		D	68

Curves Corresponding to Table VI.



In the former case the radicle was sharply curled and accompanied by very little growth of the coleorhiza. Alcohol of 30 % produced greater injury than did that of 20 %, since the majority of seedlings in lots (B), (C), and (D) showed no growth of the radicle. In a few which showed slight growth of the radicle it was sharply curved and the coleorhiza very short. Still greater injury was done by 50 %. None of the grains from which the coats were removed from the embryo side showed any growth of the radicle, and the growth of the coleoptile was very slow. In the sets (B) and (D) of the lot treated with 95 % alcohol no germination occurred, while in sets (A) and (C) the per cent of germination was high, and radicles and coleorhizas were only occasionally injured. Absolute alcohol acting for 4 hours caused little injury save where the coats were removed from the tips of kernels and from the embryo sides. Where the seedling did develop neither radicle nor coleorhiza showed injury. Chloroform proved to be less injurious than alcohol, as is shown by the fact that 68 % of the grains whose seed coats were removed from the embryo side germinated. However, among seedlings of this group there were many slowly growing radicles. A few such radicles were found among those where the coats had been cut from the tips of grains, and chloroform allowed ready access to the radicle portion of the embryo. This experiment seems to show that the covering of the grain checks the rapid penetration of chloroform and alcohol into the embryo. It also indicates that the coleorhiza and radicle are portions of the embryo which are most sensitive to the action of these agents.

That both chloroform and alcohol eventually penetrate the intact coats is evident from the following experiments. The coat and outer portions of grains which had been immersed in chloroform for 4 weeks were cut away and the remainder of the kernel when eaten gave a most decided taste of chloroform. Upon evaporating the chloroform in which corn had been immersed for some weeks oil was obtained. This oil could not have been extracted from the grains if chloroform had not entered. The following table gives amounts of oil obtained in several experiments. Approximately 300 grains of corn and 75 c.c. of chloroform were used in each case.

Table VII.

Variety of corn	Time in chloroform	Amount of oil
Abbott's White	124 days	1.635 grams
Iowa Silver Mine	30 "	.244 "
"	17 "	.075 "
High oil	30 "	.411 "
"	17 "	.246 "

As will be noted in experiments described later (page 20) the continued treatment of intact grains of corn with chloroform lowers the per cent of germination. Injury to the embryo no doubt results when a sufficient amount of chloroform has entered.

Upon immersing grains of corn in the different grades of alcohol from 10 to 80 % for a short time, 3 hours being sufficient, the liquid was taken up as shown by the grains being considerably

swollen. In this period of time much less of 90 and 95 % and little of absolute alcohol was absorbed. That alcohol as well as water passed through the coats of the grains was shown by the tests of specific gravity of the liquids before and after immersing grains in it. The grades used for this test were 10,30,50,70,90,and 95 %. 200 c.c. of each was put into glass stoppered bottles,and corn enough added so that the corn in each case was well covered with the liquid. The bottles were then sealed with vaseline. At the end of 3 hours the alcohols were poured off,tested for specific gravity by means of an alcoholometer, and returned to the respective bottles of corn. After 21 hours more another test was made.

Table VIII.

Alcoholometer Readings at 24.5° C.

Before using	After 3 hours	After 24 hours
10.50 - 11.00	11.00 - 11.50	11.00 - 11.50
30.25 - 30.75	30.00 - 30.50	30.00 - 30.50
50.25 - 50.75	50.75 - 51.25	50.25 - 50.75
69.25 - 69.75	70.00 - 70.50	70.50 - 71.00
89.75 - 90.25	90.00 - 90.50	90.00 - 90.50
95.50 - 96.00	95.00 - 95.50	95.00 - 95.50

The above table shows that the grade of alcohol was very slightly changed. The coats of the grains were probably a little more readily permeable to water than to alcohol,at least at the beginning,and the per cent of the alcohol was therefore slightly

raised. But this slight change in per cent does not at all correspond with the considerable reduction in volume of the liquid, and it is safe to conclude that the seed coats are permeable to alcohol.

Part III. To determine the effect of chloroform and of alcohol together with varying amounts of moisture.

Tables IX and X give a summary of a number of experiments that were performed with varying amounts of chloroform in air dried grains at the time of germination. Grains which had been immersed in chloroform for different periods of time were taken out and divided into lots. Some were placed directly into germinators (lot A),both with and without the addition of water. Others(lot B) were exposed to the air for various intervals,thus allowing the evaporation of certain amounts of chloroform,and then put under conditions similar to lot A both with and without the preliminary soaking in water.

Inspection of table IX A and of the corresponding curves shows that the per cent of germination decreased as the time of immersion of grains in chloroform was increased. This decline was more rapid when water was added to the grains after their removal from chloroform than when this was not done. Table IX B shows a similar decline when chloroform was allowed to evaporate from the grains. A comparison of tables IX A and IX B shows on the whole a little less rapid decrease in per cent of germination among those grains from which some of the chloroform had been removed by evaporation,especially where water was added to the grains so treated.

Table IX A.

No. of Expt.	Variety of corn	Time in		No. of grains		%
		Chloroform	Water	Used	Germinated	Germ.
1	I. S. M.	17 days	00 hr.	100	88	88
2	"	17	6	100	89	89
3	"	17	24	100	78	78
4	"	30	00	100	61	61
5	"	30	6	100	32	32
6	"	30	24	100	7	7
7	Abbott's	97	00	40	13	32.5
8	White	97	6	40	0	0
9	"	123	0	50	12	24
10	"	123	7	50	0	0
11	"	195	0	50	1	2
12	"	195	7	50	0	0

Table IX B.

No. of Expt.	Variety of corn	Length of time in			No. of grains		%
		Chlor.	Air	Water	Used	Germ.	Germ.
		days	days	hours			
13	I.S.M.	17	17	0	100	86	86
14	"	17	17	6	100	91	91
15	"	17	17	24	100	82	82
16	"	30	17	0	100	62	62
17	"	30	17	6	100	61	61
18	"	30	17	24	100	44	44
19	Abbott's	64	94	0	16	10	62.5
20	White	64	94	6	16	7	43.7
21	"	86	52	0	40	26	65
22	"	86	52	7	35	10	28.5
23	"	97	22	0	100	70	70
24	"	97	22	6	100	55	55
25	"	108	94	0	30	13	43.3
26	"	108	94	6.5	30	4	13.3
27	"	114	44	0	50	23	46
28	"	114	44	6.5	50	12	24
29	"	114	44	24	50	1	2
30	"	195	40	0	40	7	17.5
31	"	195	40	6.5	40	5	12.5
32	"	195	40	24	40	1	2.5

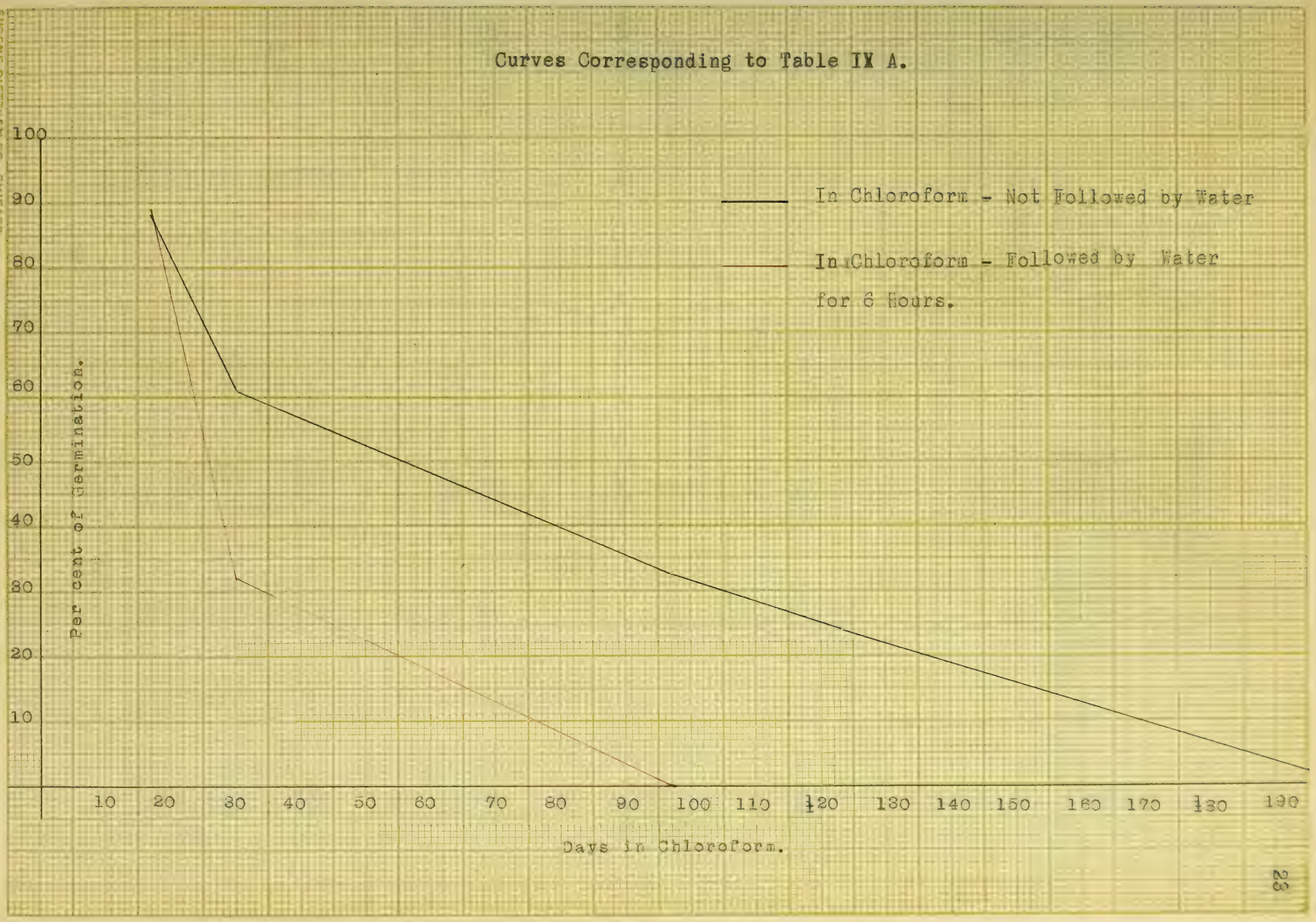

Curves Corresponding to Table IX A.
In Chloroform - Not Followed by Water
In Chloroform - Followed by Water for 6 Hours.
Per cent of Germination.
100
90
80
70
60
50
40
30
20
10
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190
Days in Chloroform.

Table X A.

Variey of corn	Time in Chloroform	Time in H_2O	No. of grains Used	No. of grains Germinated	% Germinated
I.S.M.	17 days	0 hr.	100	88	88
"	17	6	100	89	89
"	17	24	100	78	78
"	30	0	100	61	61
"	30	6	100	32	32
"	30	24	100	7	7
Abbott's	97	0	40	13	32.5
White	97	6	40	0	0
High Oil	17	0	100	87	87
"	17	6	100	86	86
"	17	24	100	54	54
"	30	0	100	79	79
"	30	6	100	56	56
"	30	24	100	54	54
"	97	0	40	31	77.5
"	97	6	40	2	5

Table X B.

Variey of corn	Length of time in Chlor.	Air	H_2O	No. of Grains Used	Germ.	% Germ.
I.S.M.	17 days	17 days	00 hrs.	100	86	86
"	17	17	6	100	91	91
"	17	17	24	100	82	82
"	30	17	0	100	62	62
"	30	17	6	100	61	61
"	30	17	24	100	44	44
Abbott's	97	22	0	100	26	26
Whits	97	22	6	100	23	23
High Oil	17	17	0	100	87	87
"	17	17	6	100	91	91
"	17	17	24	100	82	82
"	30	17	0	100	73	73
"	30	17	6	100	72	72
"	30	17	24	100	78	78
"	97	22	0	100	70	70
"	97	22	6	100	55	55

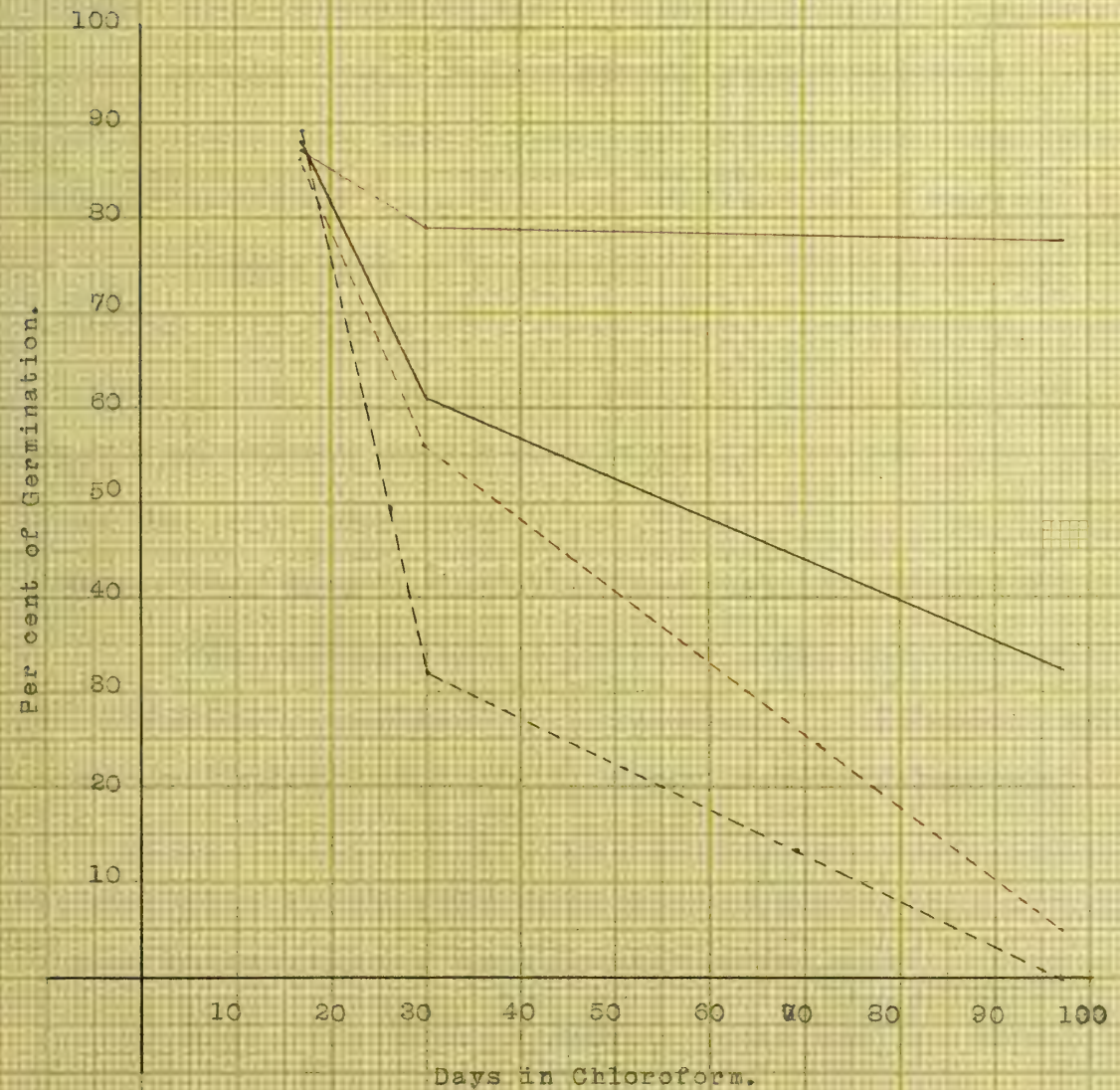
Curves Corresponding to Table X A.

——— High Oil - Chloroform not Followed by Water.

——— Low Oil - Chloroform not Followed by Water.

— — — High Oil - Chloroform Followed by Water for 6 hours.

– – – – – Low Oil - Chloroform Followed by Water for 6 hours.



Tables X A and X B give a comparison between the varieties Iowa Silver Mine and Abbott's White on the one hand, and corn of high oil content on the other. These grains were treated as indicated in table IX. The data for Iowa Silver Mine and Abbott's White was taken from table IX. Tables X A and XB show that in 17 days time the injury to the high oil corn was practically the same as that suffered by corn containing less oil. But when treated with chloroform for longer time (30 to 97 days) the high oil corn showed a higher per cent of germination, both when grains were put to germinate immediately after their removal from chloroform, and when exposed to air for some time before germination. The high oil corn treated with chloroform for 30 and for 97 days endured the addition of water after the chloroform better than did corn of lower oil content.

Experiments were performed in which grains were soaked in water for periods of 9, 6, and 3 hours, and after the removal of superficial moisture were immersed in chloroform for 3 hours. Grains so treated failed to germinate. This would indicate that the injury done when water follows chloroform is much greater than when the order is reversed (see tables IX & X).

Experiments similar to these just described were carried on using alcohol instead of chloroform. Tables XI and XII show the results under the different modes of treatment. From table XI it will be seen that allowing alcohol and water to evaporate from grains which were treated with the various grades of alcohol for 3 hours did not lessen the injury. And further that subsequent soaking in water did not affect germination as noticeably as when chloroform was followed by

water (see table IX).

Table XII shows that the taking in of water previous to the immersion in alcohol was injurious, and especially so where the higher grades of alcohol were used; that when the order was reversed the injury was not so great, though when compared with results given where water was not used, the per cent of germination was found to be somewhat lowered.

Table XI.

Corn used was Iowa Silver Mine - 50 grains for each experiment.

In agent 3 hr. Dried 7 days No water	No. grains Germ.	% Germ.	In agent 3 hr. Dried 7 days In water 8 hrs.	No. grains Germ.	% Germ.
H_2O	50	100	H_2O	50	100
10 %	50	100	10 %	50	100
20	42	84	20	42	84
30	6	12	30	10	20
40	0	0	40	0	0
50	0	0	50	0	0
60	0	0	60	0	0
70	0	0	70	0	0
80	0	0	80	0	0
90	6	12	90	10	20
95	50	100	95	48	96
Absolute	50	100	Absolute	50	100

Table XII.

Funk's Golden Dent was used - 100 grains for each experiment.

Agent	Hours in water			Agent for 3 hours			Agent for 3 hrs.
	3	6	9	Followed by H_2O for			
	Followed by			3 hrs.	6 hrs.	9 hrs.	
	agent for 3 hours						
H_2O	99	100	100	100	100	100	100
10 %	97	100	100	100	100	100	100
20	96	98	99	92	88	81	100
30	0	17	15	37	31	29	57.5
40	0	0	0	7	9	5	27
50	0	0	0	5	4	1	5
60	0	0	0	2	0	0	0
70	0	0	0	00	0	0	0
80	0	0	0	0	0	0	0
90	0	0	0	28	32	34	37
95	0	0	0	48	50	40	100
Absolute	0	0	0	46	64	48	100

IV. Discussion.

It has long been known that dormant seeds are very resistant to extremes of temperature, to severe drouth, to the action of poisons, and to conditions in general which are unfavorable for growth. Many experiments have been performed in the attempt to discover the cause of this great resistance. Brown and Escomb (1897), Thiselton-Dyer (1899), Selby (1901), Macfayden and Rowland (1902), and others, who have exposed different seeds to the extremely low temperatures of liquid air and liquid hydrogen, have found the power of germination to be little affected by such treatment. They have concluded that resting seeds retain their vitality for long periods of time in conditions where chemical changes, such as those of respiration are impossible; and that, in the absence of such chemical changes, quiescent protoplasm is in a static condition and may exist indefinitely in a state of suspended animation.

Romanes (1893) and Giglioli (1895) had already reached similar conclusions. Romanes found that certain seeds kept in a vacuum for 15 months, and others kept in tubes of oxygen, nitrogen, hydrogen, and vapors of ether and chloroform for various periods retained their power of germination. Giglioli subjected seeds to the action of certain poisonous gases and liquids, and attributed their retention of vitality to the stability of dormant protoplasm.

Dixon (1902) on the other hand, maintained that the resistance offered by dry seeds to particular poisons was due to the inability of the poison to pass through the seed coats. Becquerel (1907)

laid the marked resistance of dried seeds and fruits to absolute alcohol,chloroform,and ether to the very slow penetration of these agents through the coverings with which they are provided. Schubert (1909) carried on a long series of experiments in the attempt to discover whether the resistance seeds to poisons is due to the ability of dry protoplasm to withstand the poison, or to the acclimatization of such protoplasm to a poison when introduced slowly,or to the imperviousness of the seed coats. After many careful experiments with a number of seeds and fruits and a variety of chemical agents he concluded that in many seeds the seed coats function as a semi-permeable membrane,allowing only certain substances to pass through them;and further that resting protoplasm is unable to withstand the action of certain poisonous agents.

Brown (1907) found the grain of Hordeum vulgare surrounded by a non-living,semi-permeable covering,located in the spermoderm, which allowed the passage of water and of iodine but prevented the entrance of HCl,H_2SO_4,and the aqueous solutions of the salts of certain metals. He also demonstrated the presence of similar membranes in the grains of Avena,Triticum,and Secale. Schroeder (1911) confirmed the work of Brown on Hordeum and Triticum. He states that the portion of the coat which forms a semi-permeable membrane originates either from the inner integument or from the nucellus.

The results of the experiments on grains of Indian corn given in the preceding pages,indicate that this grain,in common with those of some other Gramineae has,in its seed coats,a membrane which when dry,is slowly permeable to chloroform and

absolute alcohol. That these agents do eventually enter and injure the grain is clearly apparent from the data given. Whether this injury is due to the inability of dormant protoplasm to withstand more than a certain amount of a poison, or to acclimatize itself with sufficient rapidity to avoid injury, or whether the embryo itself is killed as a result of asphixiation as suggested by Becquerel (1904), was not determined.

The various investigators mentioned in the bibliography agree, in the main, with Kurzwelly (1903) in that the addition of water to certain poisons greatly increases their action. Many attribute this to the increased permeability of the membrane in the presence of water. Numerous experiments have been carried on with dried and soaked seeds and with various poisons both in anhydrous form and in solution in water. Brown (1909) found that alcohol, ethylic acetate, aldehyde, and acetone when free from water would not enter grains of barley, but that in aqueous solutions they diffused readily.

Schroeder (1911) demonstrated the fact that dry grains of wheat and barley were not penetrated by certain salts, among them being KCl, KNO_3, Na_2CO_3, MgSO4, and $AgNO_3$ when dissolved in water, while $HgCl_2$, iodine, methyl and ethyl alcohols, ethyl ether, and chloroform in solution with water entered freely. The reason for this difference is by no means clear. Brown in the paper cited states that the solutions which readily penetrate through the seed coverings probably differ in some essential, but unexplained way from those which do not. He suggests the hypothesis that "some unrecognized peculiarity in the manner in which the molecules of the two classes of solutes are combined

with the molecules of the solvent water may constitute the factor which orders their different behavior with respect to the seed coverings. This hypothesis appears to be supported by the experiments which demonstrate that whereas readily diffusible solutes enter the seed together with a large amount of water, seeds placed in solution of non-diffusible solutes absorb water with some difficulty. Moreover, the observation that an aqueous solution of alcohol diffuses readily through the seed coverings which are impervious to this solute in the anhydrous state, appears to show that some form of combination of solute and water is necessary to condition diffusion of the solute through the seed coverings".

Table I on page 5 has shown the effect of absolute alcohol upon the germination of grains of Indian corn. It may be considered that absolute alcohol passes slowly through the seed coats, the rate of penetration increasing as water is added. But as seen in this table it is alcohol of 70 and 80 % which proves most injurious to these grains. Hence the amount of injury does not vary directly with the amount of water. It may be suggested as an hypothesis that the accumulation of alcohol from the lower grades is not sufficient to cause the injury done by grades of 70 and 80 % in the same time. The fact that this injury is eventually caused by the lower grades may be explained as being due to the continued action of small quantities of alcohol upon protoplasm which has taken up a large amount of water from these low grades, the decrease in germination as the per cent of alcohol is lowered from absolute may be due to the increased rate in penetration as water is added.

It will be recalled that grains of corn soaked in water for 3 hours previous to their 3 hour immersion in chloroform and in the grades of alcohol from 40 % to absolute failed to germinate. These results are similar to those of numerous investigators who have given the explanation that the thoroughly moistened covering allows the rapid osmosis of certain substances. The rate of penetration of grades as low as 10 and 20 % seems not to be influenced by the amount of water in the seed coats, there being water enough in these grades themselves to allow the maximum rate.

Inspection of tables IX and X has shown that water added to grains after their treatment with chloroform, while not resulting in the degree of injury done when water precedes the chloroform, causes a decline in the per cent of germination which varies with the time of immersion in water. The injury done where water is so added is lessened by allowing chloroform t evaporate from the grains before placing in water. Corn of high oil content not only endures prolonged action of chloroform better than corn with less oil but also suffers less injury upon the addition of water after the treatment with chloroform. These facts are doubtless of considerable importance but, from experiments completed at the present time there seems to be no adequate explanation for them.

V. Conclusions.

It is not considered that the experiments described in the preceding pages have entirely covered the problem undertaken. The necessity of using large numbers of grains in certain ex-

periments,and the difficulty of keeping these sufficiently free from moulds to avoid all interference with results,together with the unavoidable variation in temperature and moisture,make it impossible to regard the figures recorded in the data as representing absolute values. However,these experiments doubtless show the general trend of results to be given by further investigation. From the experiments completed and recorded the following conclusions may be drawn.

1. The seed coats of the grains of Indian corn furnish a membrane which is slowly permeable to ethyl alcohol and to chloroform.

2. Water increases the permeability of this membrane to the agents named.

3. Alcohol of 70 and 80 % produces injury to the embryo of corn in shorter time than do grades higher and lower.

4. The injurious effects of alcohol upon the embryo is shown by (a) delay in time of germination,(b) retardation in rate of growth,(c) weakened vitality and deformity of seedlings, (d) death of the embryo. The degree of injury depends upon the period of immersion in the liquid.

5. The coleorhiza and radicle are portions of the embryo which are most sensitive to the action of alcohol.

6. Chloroform acting upon grains for long periods of time reduces the per cent of germination. It does not injure the coleorhiza and radicle as does alcohol.

7. Water added to grains after their immersion in chloroform increases the amount of injury.

Among the questions which at the present time seem of further interest may be mentioned the following:-

1. The effect of water upon the cells containing chloroform.

2. The possible injury of certain enzymes by means of chloroform. See Grützner (1902) and Malfitano (1902).

3. The effect produced upon germination by the extraction of oil from the seed.

4. The higher resistance to chloroform offered by corn of high oil content.

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Explanation of Drawings.

The drawings on pages 41 and 42 were made from representative seedlings grown from grains treated as indicated. In each case a portion of the kernel was cut away in order to show more clearly the development of the different parts of the embryo. These parts are indicated in the drawings as follows-

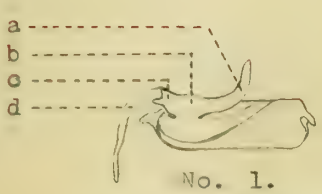
a -- coleoptile.

b -- caulicle.

c -- coleorhiza.

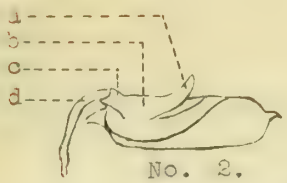
d -- radicle.

e -- secondary roots.



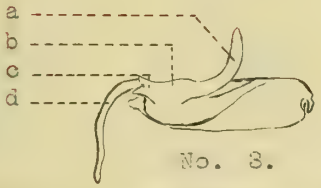
No. 1.

Water for 8 hours.



No. 2.

Chloroform for 8 hours.



No. 3.

10 % Alcohol for 8 hours.

No. 4.

10 % Alcohol for 36 hours.

No. 5.

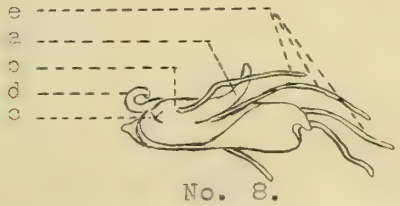
10 % Alcohol for 48 hours,

No. 6.

10 % Alcohol for 96 hours.

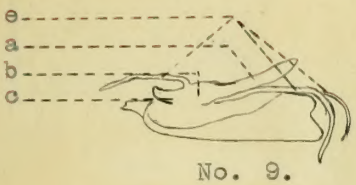
No. 7.

20 % Alcohol for 8 hours.



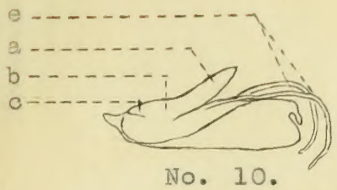
No. 8.

30 % Alcohol for 8 hours.



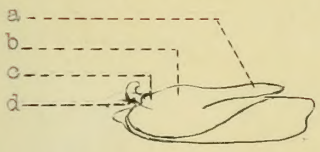
No. 9.

40 % Alcohol for 3 hours.



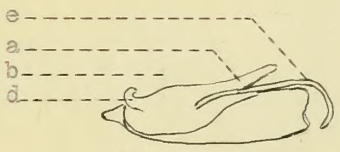
No. 10.

50 % Alcohol for 3 hours.



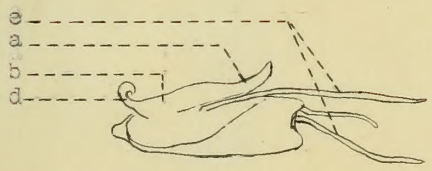
No. 11.

60 % Alcohol for 1 hour.



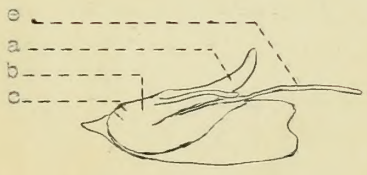
No. 12.

60 % Alcohol for 2 hours.



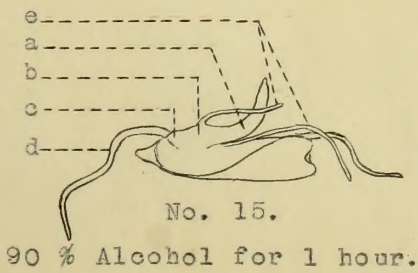
No. 13.

70 % Alcohol for 1 hour.



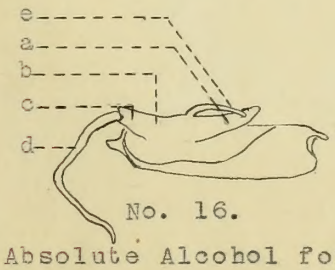
No. 14.

80 % Alcohol for 1 hour.



No. 15.

90 % Alcohol for 1 hour.



No. 16.

Absolute Alcohol for 144 hours.