SOME BIOCHEMICAL STUDIES ON SEED VIABILITY

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SOME BIOCHEMICAL STUDIES ON SEED VIABILITY

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Introduction

The object of this investigation has been to obtain a shorter method for determining viability of seeds. An abbreviated method would certainly mean a great saving of time and money. This may not be so true of seeds like pea, bean, corn, and wheat, which germinate in three or four days, but there are other seeds of economic importance which require a longer period for germination. Seeds like those of grass and celery usually require three weeks. Hybrid rose seeds may require six months for germination and the Hawthorne may require a period of from one to three years. Many other examples might be given.

There are occasions when a shorter method for determining viability may be useful even for seeds whose normal germination period is comparatively brief. Last fall in this state there was considerable rain, and cold weather began very early. The corn crop was exposed to freezing conditions and every grower was interested to know whether his seed crop had survived or not. It is quite evident then, under abnormal conditions like these, how advantageous it would be to the seed analysts to be able to shorten their seed-testing method and thus conserve both time and space.

Earlier work on this problem was terminated by Fick and Hibbard in 1924 and is reported in the former's

Master's thesis presented to the Michigan State College, as well as in a paper prepared for the Michigan Academy of Science, Arts and Letters (11).

The present work began in January 1925, when a large number of seed lots was secured for material. The problem has been developed along several lines, first taking up the method of electrical conductivity, modifying it, and finally evolving an entirely new method.

Historical

The use of electrical conductance methods for measurements in physiological research dates back to the work of Eduard Weber (1) in 1836 and du Bois Reymond (2) in 1849. Ranke (3) in 1865 studied the decrease in resistance of plant and animal tissues upon death.

Brooks (4) in 1922 worked with Laminaria, yeast, bacteria and blood cells. He showed that during the progress of heating of Laminaria the "net conductance" approached a constant value which he considered indicative of death. With Bacillus coli his results are rather variable.

Johnson and Green (38) have shown that upon death the conductivity of yeast cells increases, this being due both to exosmosis of salts and to decrease in size of cells.

The outstanding investigator in the field of electrical conductivity measurements of permeability in plant cells is Osterhout (5) who recommends this method

and shows that the results do not vary more than one percent from the mean. In a later work (6) he pointed out that the time curve expressing the increase in the permeability of Nitella during the progress of death is practically the same whether derived from measurements of exosmosis or of electrical resistance.

Many valuable contributions concerning the apparatus to be used in conductivity measurements have been made by Washburn (7), Taylor and Acree (8), Hibbard and Chapman (9) and Green (10).

Other Methods

The problem of viability of seeds has been attacked from several different points of vantage. Pierce (12,13,14) and his coworkers in 1914 noted that the heat of respiration was greater for live seeds than for dead ones. Heat measurements were made under adiabatic conditions, the seeds being placed in silvered Dewar flasks under suitable conditions for germination. He claimed that there was a "normal temperature" for each species of plant and that departures from this temperature indicated departures from the best conditions of the organism. Excess of normal indicates infection, while subnormal is indicative of loessened vigor, usually due to increased age. The author did not make any great claims as to the accuracy of this method for determining viability, but it is evident that seeds of high, low and medium viability only could be thus selected.

Lesage (15) evolved a method in which seeds were soaked in solutions of KOH of strengths varying from normal to N/682. Non-germinating seeds imparted a color to all solutions, while the viable seeds colored the strong solutions and those down to N/32, but had no noticeable effect upon weaker solutions.

Many workers have attacked the problem of seed viability from the standpoint of enzyme activity. Kastle (37) in his classical work on "Oxidases" states that peroxidases and catalases are even more widely distributed in living tissues than are oxidases.

Peroxidases attack Hydrogen Peroxide and liberate atomic Oxygen. Their presence may be demonstrated by the fact that they cause the bluing of guaicum. Samples are ground, a drop or two of guaicum added, and then two to three cubic centimeters of neutral Hydrogen Peroxide added. If the peroxidases are present the sample becomes blue, the intensity of color being proportionate to the intensity of the enzyme action.

McHargue (16) has applied this test on seeds of corn, hemp, tomato, tobacco, oats, cow peas, soy beans, castor beans and lettuce. This investigator claims that these seeds, when exhibiting zero germination, displayed no peroxidase reaction. He goes still farther and claims that the peroxidase reaction might be used for seed-testing and that seeds of high, low, and medium viability might be thus classified.

On the other hand Brocq-Rosseu and Gain (17) studied the peroxidase activity of seeds ranging from two years to five thousand years old. They found peroxidase exhibited in a wheat sample 2000 years old and claim that wheat will retain peroxidase activity 100 years after it loses its ability to germinate.

The enzyme catalase has also been employed for determining viability in seeds. This enzyme attacks hydrogen peroxide and liberates molecular oxygen. A known weight of the powdered sample is mixed with a known volume of neutral hydrogen peroxide and the volume of oxygen evolved is measured. Crocker and Harrington (18) determined the catalase activity of Johnson and Sudan grass seeds and then made additional determinations on other seeds of the same sample after they had germinated. It was found that the catalase activity increased with germination, thus paralleling respiratory intensity. With Amaranthus seeds no correlation was found to exist between catalase and respiratory intensity, vitality and age. They conclude that generally there is a close correlation between catalase activity and respiratory intensity, but not a very close relation between either and the vitality of the seeds or the vigor of the seedlings.

Nemec and Duchon (19) demonstrated a close correlation between catalase activity and viability of seeds, being able to obtain a difference between seeds varying but two or three per cent in germination. These workers employed cereal grains, legumes and other seeds.

In 1924 Marotta and Kaminka (20) found that Nemec's

catalase method could not be applied to wheat seeds. Shull and Davis (21) have found a relationship between catalase activity and delayed germination in Xanthium seeds. There is a decrease in catalase activity in delayed germination. In a later work with Xanthium seeds Shull (38) showed that oxygen accelerates germination and that a greater amount of oxygen is absorbed with the seed coats removed. Crocker (39) in 1906 showed that oxygen increases respiration and in this way initiates germination.

Since catalase is an oxidizing enzyme and believed to participate in respiration, it may be this connection with vital activities that suggested the large amount of work attempting to correlate catalase with viability.

All of the evidence herein quoted seems to furnish direct confirmation of the conception of enzymatic activity and its relation to vital phenomena as held by Palladin (22) who says: "Life processes are not to be interpreted simply as enzymatic activity". This writer continues to show how organisms might be killed without destroying the enzymes and that enzyme activity is always exhibited by freshly killed tissues where precaution was not previously taken to destroy the enzymes, and that the only difference between enzyme activity in living tissues and dead ones is that in the former they are organized in their work.

We have thus attempted to review briefly the work that has been done toward shortening the method for determining viability of seeds. The majority of the workers quoted here have sought to correlate enzymatic activity with viability.

Though enzymes are originally associated with living organisms, we have attempted to show in this historical review that they are not necessarily to be taken as indices of life in organisms.

Catalase and peroxidase might be classified as respiratory enzymes and for this reason may be thought to be concerned with vital phenomena. On the other hand both dead and live seeds respire and respiratory measurements would be of little value for determining viability. Again any carbon compound might be oxidized to carbon dioxide and the process thus be mistaken for one of respiration.

The deeper we penetrate into this problem the more complex it grows. It practically resolves itself into the question of "When is a seed dead?" or a matter of evolving a physical or chemical means for determining the difference between life and death. Has this been determinined even for the higher orgenisms? We are told that death is gradual even in man.

Whether there be a chemico-physical difference between life and death, it seems that there should be a correlation between viability and permeability of plant cells. It seems reasonable to suppose that non-living cells should be more permeable than living ones and that the salts should leach out more rapidly from the dead cells than from the live ones. The amount of salts diffusing out of the cells of the seeds should then modify the resistance of distilled water in which the seeds were soaking and this change of resistance could thus be measured electrically. An attempt was made to measure this change in resistance and to correlate it with viability of seeds. Suitable

apparatus was assembled for making the determinations.

Apparatus

The apparatus used was similar to that recommended by Hibbard and Chapman (9), there being a few minor changes. The rotary converter was discarded and the 110 alternating current from the College Power Plant used as a source of power. A variable transformer, manufactured by the Holtzer Cabot Electric Company of Boston, "stepped down" the voltage to 4.4 volts. This led directly to the bridge and galvanometer. The bridge, of the Kohlrausch roller type, was manufactured by the Leeds and Northrup Company. The wire of the bridge is 470 centimeters long with the scale divided into a thousand divisions, each division being divided into halves. The known resistance was "plug decade" type, manufactured by the Leeds and Northrup Company. This had a range of from 0.1 ohm to 20000 ohms.

The alternating current galvanometer was of the Rowland electrodynamometer type, also a Leeds and Northrup product. The determination of the balance in the resistance on the Wheatstone bridge was made by the galvanometer instead of employing the usual telephone receiver method. The stationary coil of the galvanometer is connected on the main circuit, while the swinging coil is placed across the bridge.

A telegraph key was inserted in the line between the bridge and the swinging coil. Thus the circuit could be broken as soon as the deflection on the galvanometer was noted. Since galvanometers of this type are extremely sensitive to external

fields, great care was exercised in removing the chance for error. The transformer was placed under the table and the wiring system was confined to as small a space as practicable and kept as orderly as possible.

The electrolytic cells were of the immersion type made by Eberbach Brothers of Ann Arbor from specifications furnished them. The electrodes were not platinized as was done in the work by Hibbard since it was found that small seeds would come in contact with the platinum black and possibly remove it.

Another improvement over the method as used by Hibbard and Fick was added in the form of a stirring apparatus. The seeds were poured into Pyrex beakers containing one hundred cubic centimeters of conductivity water and the beakers placed in a constant temperature water bath at 25 degrees Centigrade. A Cenco friction-drive universal motor was connected to four stirrers so that the four samples could be run simultaneously. The electrodes which were immersed in the beakers could be connected to a four-way switch.

Experimental Procedure

Beakers containing one hundred cubic centimeters of doubly distilled water were placed in the water bath, the electrodes inserted and connected, and the stirrers started. One gram of clover seeds was added to each beaker, permitting one minute to elapse between addition of subsequent samples, in order that the resistance readings might be made in the same order. For the sake of brevity this resistance which will represent that of the conductivity water plus that of the salts leached out of the seeds, will hereafter be designated as

"solution resistance". Readings were recorded at the end of 60 and 80 minutes. The results appear in table 1.

Table 1

Comparison of the germination (per cent) of clover seeds with the solution resistance for definite time periods.

Sample No.	Germination	Resistance in Ohms	
		60 minutes	80 minutes
3	92	17760	16671
5	83	18660	17172
8	75	23595	19701
11	61	23316	20112
25	57	11163	9105
24	55	8130	6360
31	53	8556	6485
28	16	14625	13557
4	7	12503	10953
29	3	18597	16296
30	1	4347	3607

From the results in this table there appears to be no correlation between viability and solution resistance, either at the end of 60 or 80 minutes. (These particular time periods were selected because Fick showed that the rate of leaching out was very uniform and that reliable readings could be taken at the end of 80 minutes.) The seeds with a germination

of from 61 to 100 per cent showed resistances of from 17000 to 23000 ohms and the 1% germination showed 4347 ohms. However the 3 and 7 per cent germination came in with resistances as high as 12000 to 18000 ohms. The resistance of the conductivity water was around 81000 ohms.

Since there appeared to be no correlation between solution resistance and viability of seeds an investigation was made on the rate of leaching out of salts from seeds. Clover and alfalfa seeds were permitted to soak in conductivity water and resistance measurements taken at regular intervals. The results, recorded in tables 2 and 3 , show that the drop in resistance is regular and continues over an extensive period of time. In fact it was later found that the salts leached out for over 24 hours.

Table 2

Clover (Samp. 24; Germ 55%)

Fall in Solution Resistance (Ohms) Produced by Seeds

Time	Resistance (Ohms)	Time	Resistance (Ohms)
20 Min	16959	62	7895
22	15340	80	6355
40	10809	81	6330
42	10385	82	6270
60	8130	83	6240

Table 3

Alfalfa

Fall in Resistance of Solution Produced by Seeds

Time	Resistance (Ohms)	Time	Resistance (Ohms)
20 Min	12153	220 Min	3726
40	9501	240	3636
60	8070	260	3537
80	6741	280	3438
100	5547	300	3384
120	4875	340	3288
140	4425	360	3237
160	4143	380	3060

The question arose as to whether it was absolutely essential to employ conductivity water. Accordingly an experiment was conducted in which the rate of diffusion of salts was determined for two samples of seed, one in ordinary distilled water and the other in conductivity water. The results appear in Table 4.

Table 4

Comparison of Distilled Water with Conductivity Water For Measuring Solution Resistance (Alfalfa Seeds)

Distilled Water	Time	Conductivity Water
6038 Ohms		81000 Ohms
5615	2 min	19470
5365	4	13740
5120	6	12750

Table 4 (Continued)

Distilled Water	Time	Conductivity Water
4798 Ohms	8 Min	12375 Ohms
4990	10	11850

The resistance of the conductivity water is much higher than that of the distilled water yet both are lowered by the introduction of the seeds and both continue to drop regularly. Also the figures given by the distilled water offered a better working level.

In order to verify original results a new supply of seed samples was secured, sending to several different states for supplies. The original methods were employed: one hundred cubic centimeters of conductivity water, one gram of seeds stirred in solution and readings in resistance taken regularly. The results appear in Table 5.

Table 5

Comparison of Germination (Clover) to Solution Resistance(Ohms)

Sample	Germination	Resistance	Original Resistance	Fall in Resistance
40	0%	28610 Ohms	65190	36580
42	1	17320	72640	55320
30	1	11050	64070	53020
35	2	24010	72640	48630
54	2	21550	74750	53200
36	3	17620	81740	65120
29	4	23500	75470	51970

Table 5 (Continued)

Sample	Germination	Resistance	Original Resistance	Fall in Resistance
34	4	39500	70650	31150
37	4	30490	75470	45100
51	4	54520	80910	26390
32	6	14450	75470	61020
48	6	35250	71970	36720
4	7	23330	76960	53630
45	11	39020	91010	51990
47	12	21950	75470	53520
28	17	25460	75470	50010
52	19	20400	72640	52240
43	22	23330	89010	65680
46	22	13200	76210	63010
49	30	6026	76210	63010
26	41	25090	70000	63974
6	52	42630	91010	67680
10	53	18330	80910	38280
31	55	19150	77720	59390
24	56	24250	82590	63440
53	58	24250	85240	60990
44	59	13640	76960	65640
9	61	33670	76210	42540
11	61	34840	77720	42880
25	61	34050	86150	52100
39	62	5314	58500	53300
38	70	21450	74750	53300
8	75	68740	81740	13000
5	83	31150	92040	60890
7	83	19070	74750	55680
3	92	25460	74750	49290

It will be noticed from table 5 that still no correlation between viability and resistance is found. In this case the test has been applied to 36 samples.

It was noticed that there existed a slight difference in the "specific conductivity" of the different electrode cells. Though the difference was not great, it was feared that this might be the source of some greater or inexplicable error. Hence the same electrode was employed on nine different samples of clover. The results will be found in table 6.

Table 6

Solution Resistance of Clover Seeds Compared to Germination. Same Electrode on all Readings

Sample	Germination	Solution Resistance (15 Min.)	Resistance of H_2O	Fall in Resistance
30	1 %	11550 Ohms	15640	4090
35	2	22790	38780	15990
36	3	11880	41550	29670
29	4	23900	42630	18730
4	7	25840	42630	16790
45	11	24970	40250	15280
47	12	10660	49880	39220
28	17	21450	50240	28790
52	19	16880	51350	34470

From the results in table 6 it is again evident that there is no correlation between resistance and viability, there being high and low resistances for samples of all viabilities.

Thue far red clover (Trifolium pratense) has been most frequently employed in the work. It will be recalled that this particular type of seed possesses a hard seed coat and for this reason salts may not diffuse out very readily. To surmount this difficulty the seeds were scarified in H_2SO_4 (sp. g. 1.82) for fifteen minutes. They were next washed thoroughly with tap water and finally rinsed three times in distilled water. Three minutes after removal from the H_2SO_4 the seeds were plunged into distilled water preparatory to determining solution resistance. The results are presented in table 7.

Table 7

Solution Resistance of Clover Seeds Scarified With H_2SO_4

Time	Solution Resistance	
	Sample 3 (Germ. 92%)	Sample 4 (Germ. 7%)
25 Min.	12370 Ohms	13360 Ohms
27	11880	12520
30	10920	10960
32	10370	10080
35	9455	8975
40	8051	7513

The resistance of the scarified samples (92% and 7% germination) were almost identical despite the great difference in germination. It is possible that fifteen minutes was not long enough to thoroughly scarify clover, yet the seeds germinated much more readily after the treatment with H_2SO_4.

In all of the readings, whether the same electrode was used or not, there was a slight variation in the duplicates of the same sample. Hence an experiment was conducted in which 10 replicates of the same sample were run, this time employing timothy seed. Otherwise the usual procedure was followed. The figures for resistance in table 8 represent the average of ten readings. In these results the average solution resistance for all samples is surprisingly similar. The lowest is 3875 ohms and the highest is 7118 ohms and show no correlation.

Table 8

Comparison of Germination (%) of Timothy Seed With Solution Resistance (Ohms)

Sample	Germination	Solution Resistance (Average of ten)
67	84%	5917 (After 25 min.)
58	81	7113 "
57	54	6007 "
63	12	3875 "
70	0	5625 "
65	0	5181 "

In all of the experiments thus far described the permeability or change in resistance has been measured by the egress of salts, the seeds having been immersed in distilled water. Most other investigators in this field have employed salt solutions in place of the water for the measurement of permeability of living and dead cells.

Osterhout (23) , in some of his work, found that salts could be divided into two classes according to their effect upon conductivity of cells: (1) salts (bivalent and trivalent cations) that produce a rise in resistance, followed by a fall; (2) those (monovalent cations) that produce only a fall in resistance. When the tissue is placed in artificial sea water in which NaCl has been replaced by LiCl, the LiCl molecule penetrates the cell and the resistance is lowered.

An attempt was made to determine the variation in solution resistance when the seeds were placed in solutions of electrolytes of known strength. Two samples of timothy seed were placed in N/10 solutions of LiCl and the resistance measured at regular intervals. The results may be found in table 9.

Table 9

Comparison of Germination of Timothy With Solution Resistance in N/10 LiCl

	Resistance in Ohms	
(LiCl)	48.81	47.28
Time	Sample 57 (Germ. 54%)	Sample 65 (Germ. 0%)
10 Min,	47.71	49.01
15	47.28	48.00
20	47.28	48.23
25	47.49	47.46

The resistance was not only similar for both samples,

but remained practically the same throughout the experiment for both the 0 % and the 54 % germination. It is probable that the solution was too strong. Experiments were performed with greater dilutions (N/100) but with similar results.

The LiCl solution was next replaced by solutions of KOH. It will be recalled that Lesage (15) employed this alkali for testing seeds, claiming that the viable seeds differed from the non-viable ones in their inability to color solutions of KOH whose strength was less than N/32. It was thought by the present writer that this behavior of the seeds toward KOH might be correlated with permeability of the cells and it was this which occasioned the following experiment.

One gram of timothy seeds was added to 100 cubic centimeters of N/800 KOH solution, as in the preceeding experiment, and the beakers placed in a water bath at 25 degrees Centigrade. The results appear in table 10.

Table 10

Comparison of Germination of Timothy

With Solution Resistance of N/800 KOH

Time	Sample	Germination	Solution Resistance
	KOH		731.5
10 Min.	58	81 %	733.5
25	"	"	750.0
	KOH		687.5
10	57	54	702.0
25	"	"	719.5
	KOH		673.5

Table 10 (Continued)

Time	Sample	Germination	Solution Resistance
	KOH		673.5 Ohms
10 Min.	61	6 %	798.5
25	"		847.5
	KOH		647.0
10	65	0 %	660.0
25	"		673.5

The samples represented germinations of 0, 6, 54, and 81 % yet the resistances varied only from 673.5 to 847.5 ohms for 25 minute readings. There was no correlation between viability and solution resistance.

Since our samples varied among themselves, an attempt was made to compare the living and dead cells of the same sample. Osterhout (6) showed that the time curve expressing the increase in permeability of cells of Nitella during the progress of death is practically the same whether derived from measurements of exosmosis or from electrical resistances. He derived the constant from the formula $K = \frac{1}{T} \log \frac{a}{a - x}$ where $\underline{a}$ = total amount of chlorides and $\underline{x}$ = the amount diffusing out in the time $\underline{T}$.

Samples of the same seed were divided into two lots. Lot 1 was run through the usual routine of conductivity measurements. In lot 2 the samples were first killed by dry heat (90 to 100 degrees for 5 days) and then run.

It has also been suggested that foreign substances might adhere to the seeds and thus alter the conductivity of the solutions. To obviate this difficulty the seeds were first washed by spreading on a filter and pouring distilled water through them. Table 11 includes the results of both these procedures.

Table 11

Comparison of Viability and Solution Resistance of Timothy. Seeds Living and Dead; Washed and Untreated

Samp.	Germ.	Normal Seeds		Killed Seeds	
		Untreated	Washed	Untreated	Washed
57	54 %	6129	6313	5761	10830
57	54	5512	7857	4881	9380
58	81	8215	15320	7073	13420
58	81	7007	12600	5937	12830
61	6	6340	12620		20770
61	6	6722	12080		17030
64	0	4045	12080		9608
64	0	4025	11010		7731
65	0	3245	17250	5301	10750
65	0	3351	16040	4478	9493
67	84	10080		5617	11740
67	84	9418		5083	10120
70	0			5824	
70	0			7823	

Reference to table 11 indicates that washing increases the resistance but there is still no correlation between

resistance and viability either before or after washing. Killing the seeds did not seem to alter the resistance of the solutions.

At this point the method was modified in such a way that resistance was measured by actual contact with the seeds. The immersion electrode cell was replaced by a glass cylinder containing copper pistons for electrodes, one of these pistons being adjustable. The cylinder was placed in an upright position, the adjustable piston removed, and one gram of seeds poured in. One cubic centimeter of distilled water was added, the piston replaced, and the electrodes connected to the Wheatstone bridge.

The cylinder was 1 centimeter in diameter and about 10 centimeters long. The piston was permitted to rest upon the seeds in the cylinder by its own weight. Samples of 0 and 84 % germination were used and the readings recorded at intervals of 19, 35, and 50 minutes. There was no significance in the difference in the resistances of the two samples. See table 12.

Table 12

Comparison of Viability and Resistance of Timothy by Contact

Time	Sample	Germination	Resistance
19 Min	65	0 %	4091 Ohms
19	67	84	2645
35	65	0	2987
35	67	84	2299
50	65	0	2704
50	67	84	2407

A number of references has been made to the work on enzymatic activity as related to viability. Kastle (37), in his excellent monograph on Oxidases, mentions the fact that catalases, which occur in plant extracts and which liberate molecular oxygen from hydrogen peroxide, will reduce other oxidizing agents, among them being $KMnO_4$. While making some preliminary tests for catalase it was noticed that seeds reduced very dilute solutions of potassium permanganate at different rates. The thought occurred that the substances leaching out of seeds might be organic in nature and hence not measurable by conductivity methods. If this were true the distilled water might be replaced by $KMnO_4$ solution and the change in resistance noted as the permanganate is reduced.

Accordingly experiments were set up in which the reduction of $KMnO_4$ by seeds could be measured electrolytically. The strength of the $KMnO_4$ was M/20000. Timothy, as well as larger seeds, were employed. The quantities used were one gram of timothy, 50 peas, or 100 wheat seeds. Resistance was determined as in the original solution methods, readings being taken at the end of 25 minutes. The results are recorded in tables 13, 14 and 15.

Table 14 (Continued)

Sample	Germination	Solution Resistance	Average
109	83 %	9048 Ohms	9729
"	"	10410	
103	76	10660	10535
"	"	8051	
105	75	11830	10072
"	"	8315	
104	63	5699	5518
"	"	5337	

Table 15

Comparison of Viability of Timothy Seed

With Solution Resistance in $KMnO_4$ (M/20000)

Sample	Germination	Solution Resistance	Average
58	81 %	6340 Ohms	6340
68	70	4970	4795
"	"	4620	
57	54	5974	5715
"	"	5456	
65	0	4205	4266
"	"	4327	

Table 13

Comparison of Viability of Wheat With

Solution Resistance in $KMnO_4$ (M/20000)

Sample	Germination	Solution Resistance	Average
108	86 %	7361 Ohms	7361
101	86	7364	7364
100	81	7224	7292
100	81	7361	
102	22	4663	5119
102	22	5576	

Table 14

Comparison of Viability of Peas With

Solution Resistance in $KMnO_4$ (M/20000)

Sample	Germination	Solution Resistance	Average
110	97 %	12880 Ohms	12985
"	"	13090	
106	93	16250	15945
"	"	15640	
107	93	16320	16145
"	"	15970	
111	92	16320	13355
"	"	16390	
112	90	11600	10800
"	"	10000	
108	86	7361	7361

Table 16

Comparison of Viability of Wheat With

Solution Resistance in $KMnO_4$ (M/20000)

Sample	Germination	Solution Resistance	Average
117	98 %	7007 Ohms 6340 5823 5798 6340 6807 5873 5748	6217
113	79	5723 6026 5480 5314 5552 7065 5408 5576 5974 5376	5749.4
116	13	6892 7153 8484 8698 7483 7046 7857 8762 7391 7391	7713.9

Greater encouragement was received from the resistance measurements in $KMnO_4$ than from any thus far. In table 13 the wheat shows true correlation. The viability varies directly with the resistance. In the case of the peas (table 14) the samples of from 90 to 97 % germination show high resistance. With but one or two exceptions the rest of the figures for resistance fall in their proper places in the table. The second experiment with wheat (table 16) and the one with timothy (table 15) are not entirely in accord with the first results.

The same procedure was employed with corn seeds except that three different temperatures (15, 25 and 35) were maintained in the water bath while the conductivity readings were being made. The results appear in table 17 and indicate as much correlation between solution resistance and viability as did those with the peas.

Table 17

Comparison of Viability of Corn With Solution Resistance in $KMnO_4$ (M/20000)

Sample	Germination	Solution Resistance		
		15 degrees	25 degrees	35 deg.
155	100 %	17030 Ohms		10160
156	99	18410	14940	12940
157	98	12120		8083
158	96		12220	
152	96	12570		3021
160	93		8083	
150	94	8149		5601

Table 17 (Continued)

Sample	Germination	Solution Resistance		
		15 degrees	25 degrees	35 deg.
159	79 %		4490 Ohms	
148	70	13470	7668	7212
144	60	8315		
154	1	4684	3351	3021
149	0		5480	

Next corn seeds were divided into two lots. Lot 1 was placed on a window ledge over night when the temperature was 0 degrees Fahrenheit. (Subsequent tests showed that these seeds did not freeze. Hence they will be used as checks against the killed seeds).

Lot 2 was kept in an air oven at 60 to 130 degrees Centigrade over night. These seeds were killed by the process. The results appear in table 18.

These results reveal the fact that heating lowered the resistance slightly. In the samples 150 and 144 the grains were browned by the heat treatment and the resistance was naturally much lowered. There is about as much correlation between viability and resistance evinced in this case as was true of the pea experiments. All of the samples in the 90 to 100 % germination class as a whole rank high in resistance, extending from 13,980 ohms to 31,150 ohms, though not always in the proper order among themselves. Samples 148 and 144, which respectively represent 70 and 65 % germinations, are

reversed in order though both are lower than those of the 90% germination class. Sample 154, germination 1 %, was the lowest in resistance, being 6449 ohms.

Table 18

Solution Resistance of Live and Dead Corn Seeds in $KMnO_4$

Sample	Germination	Solution Resistance (50 Min.)	
		Lot 1 (Alive)	Lot 2 (Dead)
155	100 %	19670 Ohms	17395
156	99	27410	24680
157	98	17250	14760
152	96	16810	15000*
150	94	14650	12385**
148	70	12170	12980
144	65	14920	10556**
154	1	6449	5712

* Duplicate discarded. **Browned by heat.

At this point a deviation from the usual method of procedure was made. Instead of measuring the solution resistance of the seeds, a few experiments were performed to determine the rate of moisture intake and rate of dessication of seeds.

Atkins (24) has shown that the absorption of water by living and dead seeds is the same until germination commences. Shull (25) claims that selective semipermeability is a phenomenon of the seed coat and not related to vital processes.

Crocker and Harrington (18) remind us of the suggestion that the gradual loss of viability of air-stored seeds with age is due to time denaturing or time coagulation of embryo proteins. Hence an attempt was made to induce the seeds to absorb water from a moist atmosphere and to measure the degree of absorption. Also the time rate of dessication was determined.

Air - dried seeds (clover) were placed over H_2SO_4 (C.P.) in a dessicator and weights computed at regular intervals, until the seeds stopped losing weight. The figures in table 19 indicate the percent of moisture loss based on the original weight.

Table 19

Comparison of Viability of Clover Seeds to Water Loss

Sample	Germination	Loss of Water (Based on Original Air-Dry Weight)	
4	7 %	7.24 %	7.09 (Av)
"	"	6.95	
10	53	9.59	8.40
"		7.21	
5	83	7.28	8.23
"	"	9.18	

Though the duplicates are not close in the results in table 19, the percent of water loss was nearly the same for all samples, irrespective of their viability.

The rate of moisture absorption was determined by placing air - dry timothy seeds in a chamber in which the air was saturated with moisture. Weighings were made at

regular intervals. At the end of twenty-four hours the seeds were placed in a dessicator over sulfuric acid and kept there for an additional twenty-four hours to note the rate of water absorption again. The results appear in table 20.

Table 20

Comparison of Viability of Timothy Seeds to Moisture Absorption

Samp.	Germ.	Percent of Water Absorption Figured on Air-Dry Weight				Percent of Loss (Per air-Dry Wt.)
		1 hr.	3 hr.	12 hr.	24 hr.	24 hr. (H_2SO_4)
64	0%	1.07	2.84	8.91	12.07	3.66
65	0	.92	2.68	8.42	12.91	2.99
57	54	.615	2.30	7.74	11.7	3.13
68	70	.935	2.82	8.50	13.41	2.79
58	81	.86	2.44	7.30	11.52	3.39
67	84	.997	2.76	8.37	12.80	3.42

The degree of water absorption was uniform for samples of various viabilities, as was true of the figures for dessication in the previous experiment. There was no difference where the seeds were re-dessicated; that is, they lost water in the usual manner.

Discussion

Evidence has been presented in the first part of this paper indicating that there is no correlation between viability of seeds and the electrical resistance of solutions of these seeds or "solution resistance". Why is it not possible to develop a conductivity method for determining viability of seeds ? A number of reasons might be advanced.

(1) The method, as applied, presupposes that the mineral content is the same for different samples of seeds of the same species and that the change in solution resistance would be dependent solely upon the change in permeability in the seeds.

(2) Brooks (4) eliminated the individual difference factor by employing the same plant for measurements of both live and dead cells. He developed the terms "net conductivity" and "dead conductivity".

(3) Another possible reason for the failure of a conductivity method might be found in the chance for minerals to adhere tenaciously to the exterior of the seed coats, their presence being due to previous conditions of handling.

(4) In practically all of the literature citations on electrical conductivity measurements of permeability, the actual tissues were measured rather than solutions.

Actual contact methods were attempted in this work with both whole seeds and with the powder. However, an adequate type of electrode has not as yet been developed to suit the problem. It might be possible to use disks of the

larger seeds, following the routine of Osterhout (23), but we are then deviating from the path of practicability. The work with potassium permanganate and conductivity seemed the most promising as far as correlation was concerned. Though there were a few discrepancies, resistance for the most part rose with viability. Curves plotted from any of the individual tables indicate as great a degree of consistency as most workers seem to obtain from other methods. Seeds of high viability repeatedly exhibit a proportionately high resistance in solution.

Why should potassium permanganate solutions furnish better results than distilled water ? It is possible that the substances leaching out of the seeds and which are indicative of viability might be non-electrolytes. Say, for instance, that they are readily oxidizing substances of organic nature, such as amines, and that the oxidation would be brought about by the potassium permanganate. The resistance would then be lowered in proportion to the amount of $KMnO_4$ reduced.

The reduction of the permanganate takes place as a result of soaking the seeds in the solution. It has been shown that soaking seeds hastens germination only because the imbibition of water is the first step in germination. Now the initial chemical change in the process of germination involves enzymatic activity. Could it not be possible that in soaking the seeds, the first step in germination is begun and the byproducts of the enzymatic processes are leached out.

Again, with the entrance of water in the seeds, we might have new products formed by hydrolysis, other than those hydrolyzed by the enzymes.

On the other hand we must not attempt to seek one method that is applicable to all seeds. We cannot apply one method of sterilization, germination or cultivation to all seeds, for they are embryos of vastly different kinds of plants. Also we have carbohydrate, fatty, and proteinaceous seeds and this alone should be responsible for different chemical behavior.

Summary

(1). The literature dealing with the application of electrical conductivity measurements in living and dead cells is briefly reviewed.

(2). Recent methods for determining viability of seeds are enumerated.

(3). The electrical conductivity method for determining viability of seeds is attempted.

(4). A description of the apparatus employed is presented.

(5). No correlation was found between viability of clover seeds and solution resistance (resistance of solution in which the seeds were soaked).

(6). Experiment showed that the resistance of water in which clover seeds were soaking continually dropped, even after twenty-four hours.

(7). Distilled water was substituted for conductivity water (doubly distilled water), similar results being obtained.

(8). Experiments were repeated, using a larger number of samples, noting the original resistance of the water and the drop in solution resistance after addition of seeds. No correlation between viability and solution resistance could be found.

(9). No better results were obtained by first scarifying the seeds or by using the same electrode for all determinations.

(10). Timothy soaked in salt solutions (LiCl) lowered the resistance as when soaked in pure water. In acids and bases the resistance increased.

(11). Live seeds were compared with seeds of the same sample which had been artificially killed. Killing seemed to lower the resistance.

(12). Timothy seeds were rinsed with distilled water before adding to solution. Washing the killed seeds raised the resistance but exhibited no marked effect on the live ones.

(13) An improvised method making actual contact with timothy seeds revealed no uniform results.

(14). A temporary departure from the original method was made. A study was made of the tendency of seeds to give up moisture in a dry atmosphere and to absorb moisture from a moist atmosphere. No correlation with viability was noted.

(15). The conductivity method was resumed. Determinations were made of the solution resistance of timothy

wheat, peas, and corn in dilute potassium permanganate solutions. Greater indications of correlation between viability and solution resistance were obtained than in any of the previous experiments.

(16). Corn, soaked in potassium permanganate at 15, 25, and 35 degrees Centigrade, produced no new data.

(17). Killing corn seeds seemed to have no marked effect upon the resistance measurements in $KMnO_4$.

Reduction of Potassium Permanganate by Viable and Non-Viable Seeds

In determining the solution resistance of seeds in potassium permanganate it was noticed that the permanganate was reduced at different rates by different samples, the end-point being an easily-recognized amber color. Resistance measurements taken at regular intervals did not take into account the color changes, being concerned only with the change in solution resistance.

The method was accordingly altered in such a way that the time rate of reduction of $KMnO_4$ by the seeds could be measured. One hundred corn seeds were ground to the fineness of meal, one-gram samples weighed out and permitted to stand in twenty cubic centimeters of distilled water for an hour. At the end of this time the mixture was filtered, one cubic centimeter of the filtrate drawn off and added to 1/2 cubic centimeter of M/800 $KMnO_4$. The time when adding to the permanganate was recorded as well as the time when it was completely reduced.

The seeds were ground because the substances reducing the permanganate were dissolved in the water more readily and the process thus hastened. The cells would hardly be destroyed by this coarse grinding since it was performed in a meat chopper. Usually material must be ground with quartz sand in order to rupture the individual cells. Grinding did not influence the relative rates of reduction by the different samples. The results will be found in table 21.

Table 21

Relation of Viability of Corn Seeds to Time Rate Of Reduction of $KMnO_4$ by Aqueous Extracts (Powder)

Sample	Germination	Time to Reduce
122	98.3 %	38.3 Min.
124	97	30.8
129	96	40.2
128	87.5	36.7
127	87	29.8
126	80.4	26.8
123	34	13.3
121	33	13.0
125	3	5.9

It was found that, with the exception of but a few of the high-germination samples, there was direct correlation between viability and time of reduction of permanganate. The higher the germination the longer the time required for complete reduction of the permanganate.

A larger number of samples was secured and the corn meal was passed through a twenty-mesh sieve before soaking. As was true in other experiments, where a greater number of samples was employed, the results are not nearly so uniform. These results are presented in table 22.

Table 22

Relation of Viability of Corn Seeds to Time Rate of Reduction of Potassium Permanganate by Aqueous Extracts of Meal(20 Mesh)

Samp.	Germ.	Time to Reduce	Samp.	Germ.	Time To Reduce
139	100 %	21.9 Min.	150	94	15.9
142	100	18.5	153	93	15.5
156	99	16.5	160	93	14.1
146	99	10.9	143	83	17.9
147	99	14.3	159	79	23.5
140	99	14.2	148	70	16.5
157	98	12.5	141	71	10.3
152	96	22.5	138	65	18.3
158	96	17.9	144	65	10.9
151	95	21.9	154	1	
145	94	12.0	149	0	7.5

Grinding the seeds and extracting the powder is a rather intricate process for a simplified method. An attempt was made to use the whole seeds. Ten seeds of corn were placed in twenty cubic centimeters of N/1000 potassium permanganate and the time recorded. The time was again recorded when the permanganate was completely reduced. It was found that if a few drops of N/10 oxalic acid were added to the mixture the end point would be clear and colorless instead of the amber color. The results may be found in table 23.

Table 23

Relation of Viability of Corn to Time Rate Of Reducing Potassium Permanganate(Whole Seeds)

Sample	Germination	Time To Reduce
152	96 %	22.5 Min
151	95	21.9
146	99	10.9
143	83	17.9
141	71	10.0
149	0	7.5

In the six samples included in table 23 there is only one (146) which does not occur in the regular order. There is here almost complete correlation between viability and the reduction of potassium permanganate.

To hasten the time of reaction the seeds were first soaked over night in water and one cubic centimeter aliquots withdrawn for the test instead of using the seeds. One cubic centimeter of this extract was treated with one drop of N/2 $KMnO_4$. The time required for complete reduction was recorded. The results will be found in table 24.

Here, too, low viability seemed to be consistent with the rapidity of reduction of potassium permanganate, although there was a great amount of non-uniformity in the table. The results in table 25 were obtained in the same manner as those in table 24. Here again the low-germinating seeds were first to reduce the potassium permanganate.

Table 24

Relation of Viability of Corn Seeds to Time Rate of Reduction Of Potassium Permanganate by Aqueous Extracts of Whole Seeds

Samp.	Germ.	Time To Reduce	Samp.	Germ.	Time To Reduce
155	100 %	23 Min	167	81 %	20.5
161	99	23	168	65	19
162	99	21.5	171	25	12
163	98	24.5	173	2	7
172	97	11.5	169	0	18
164	86	24.5	170	0	12
166	83	21.0			

Table 25

Relation of Viability of Corn to Reduction Of $KMnO_4$ by Aqueous Extracts of Whole Seeds

Sample	Germination	Time To Reduce
162	99 %	39 Min
163	98	34
164	86	38
166	83	37.5
167	81	29.5
168	65	31
170	0	22
149	0	12
154	0	19
169	0	22

At the conclusion of this experiment on reduction of potassium permanganate some additional experiments were conducted with beans. The results (table 26) exhibit a moderate amount of correlation. The figures for germination were obtained by selecting fifty normal - appearing seeds, sterilizing and germinating. This was done because it was later learned that these were not true samples but had been more or less adulterated by the growers. This is quite evident from the results of several different germinations. In one set a hundred seeds were selected at random; in another the twenty seeds that had been used in the reduction of the $KMnO_4$ were subsequently germinated. The variation may also be due to the difference in methods. See table 27.

Table 26

Relation of Viability of Bean Seeds

To Time Rate of Reduction of $KMnO_4$

Sample	Germination	Time to Reduce
174	100 %	31 Min.
175	100	31
176	98	25.5
177	98	26.5
178	100	19
179	46	38
180	0	14

Table 27

Experiment on Germination

Sample	Percent Germinating		
	100 Seeds	20 Seeds	50 Seeds (Selected and Sterilized)
174	90	100	100
175	89	95	100
176	85	85	98
177	76	90	98
178	27	65	100
179	15	15	46
180	0	0	0

The next experiment was with the same samples.

As will be shown in the latter part of this paper the reducing substance has been found to be present in large quantities in the seed coat of the bean. Accordingly ~~these~~ the same samples were soaked over night in distilled water, the seed coats removed and placed in the permanganate solutions. Ten seed coats were placed in 10 cubic centimeters of N/1000 $KMnO_4$ and the time for reduction recorded. These results will be found in table 28. The ten seeds, after the coats had been removed, were germinated. Not only was direct correlation between viability and rate of reduction found, but sample 179, which formerly did not fit into the proper order, appeared in its proper place in the table.

There is a correlation between vitality and rate of reduction.

Table 29

Relation of Viability of Beans To Time Rate Of Reduction of $KMnO_4$ by Their Seed Coats

Sample	Germination	Time to Reduce
174	100 %	26 Min.
175	100	24
176	100	23
177	100	23
178	90	20
179	50	16
180	0	1

Table 30

Relation of Viability of Beans to Time Rate Of Reduction of $KMnO_4$ by Their Seed Coats

Sample	Germination	Time To Reduce
174	100 %	14.3 Min
175	100	14.6
176	100	16.3
177	100	16.0
178	97	15.3
179	73	11.3
180	0	0.58

The results in table 30 were obtained in the same manner as those in the preceeding table. The same seeds were germinated after the seed coats had been removed for the reduction experiment.

An experiment was next performed in which the three parts of the seed (seed coat, cotyledons, plumule and hypocotyl) were treated with the potassium permanganate. In this case there were employed 5 seed coats, 10 cotyledons, and 10 embryos. The figures for germination are those obtained in selecting and sterilizing 50 seeds. The results in table 31 show almost complete correlation in all cases. The seeds germinating above 90 percent do not always occur in the proper order yet they are usually higher in the table than those of low germination.

Table 31

Relation of Viability of Beans To Time Rate of Reducing $KMnO_4$ by Different Parts of Seed

Sample	Germination	Time Required For Reducing		
		Coats	Cotyledons	Embryos
174	100 %	34 Min	11	12
175	100	30.5	6	11
176	98	27.5	8.5	9
177	98	32.5	6.5	8.5
178	100	30	7	10
179	46	26.5	5	7
180	0	1.5	2	3

Discussion on Reduction of Potassium Permanganate

From the results presented in this paper it is obvious that seeds of low viability exhibit a tendency to reduce potassium permanganate in less time than is required by seeds of higher viability. It does not matter whether the seeds of zero germination have been killed by heat, frost, disease, or have died of old age. They are nearly always the first to reduce the permanganate. It is possible that this method might be employed for determining seeds of high, low and medium viability, but, like other methods, mentioned above, it needs considerable refinement before it will show differences in seeds varying 5 or 10 percent in germination.

It is of interest to know the nature of this substance which reduces the permanganate. Reed (26) believes that the peroxidases in plant juices, having the power to absorb oxygen from oxygenases, will attack $KMnO_4$ in the same manner. Bunzel and Hasselbring (27) note that $KMnO_4$ may be reduced to a straw color by peroxides of manganese and, further to a clear solution by organic substances, but they hold that the oxidations are brought about by peroxides of manganese rather than by activated plant peroxidases.

That the reduction mentioned in this paper is not enzymatic may be demonstrated by first boiling the aqueous extract of seeds for fifteen minutes. The reduction will take place just as readily after boiling.

There is evidence supporting the view that these reducing substances might belong in the group of peptides, acid amides, and amino acids. If the proteins are precipitated by lead acetate and the excess lead removed from the filtrate by means of sodium carbonate, the filtrate will still reduce the potassium permanganate. Furthermore, an aqueous extract of the precipitate brought down by the lead acetate will not produce the reduction unless boiled with 10 % HCl or incubated with pepsin solution.

Proteoses would remain in the filtrate after the proteins had been removed with lead acetate. However, phosphotungstic acid brought down no precipitate and it was assumed that in this case there were no proteoses present.

For a long time it was believed that ungerminated seeds contained no protein cleavage products. This view has now been changed by Jodidi and coworkers (28), who have found amino acids and peptides to be present in ungerminated kernels of maize, rye, wheat and oats. Bushey (29) showed that frosted and "hailed" corn, besides being high in certain proteins, was also much higher in amide content than normal grains.

Summary

(1) Another method for determining viability of seeds is suggested. The method is based upon the relative time required by seeds of different viability for reducing dilute solutions of potassium permanganate.

(2) The method has been used on corn and beans and as yet is not recommended for other seeds.

(3) This method is suggested for use in selecting viable from non-viable seeds. Probably greater refinement of the method will permit its use for selecting samples of smaller differences in viability.

(4) Proof is given that the substance which reduces the permanganate is not an enzyme and that it might belong in the group of substances known as amino acids, peptides, and amino acids.

AQUEOUS EXTRACTS OF SEEDS AS AGENTS IN THE PREPARATION OF SILVER SOLS

Another phase of this problem developed in this work was the property of reducing molecular silver to the colloidal phase as exhibited by aqueous extracts of seeds. This peculiar property of the extracts was discovered while testing for chlorides. Silver nitrate had been added to the solutions in which the seeds had been soaked and the solutions accidentally permitted to stand over night. In the morning a dark brown color, characteristic of silver in the colloidal phase, had appeared in the solution.

Many methods have been suggested for the preparation of colloidal metals by condensation with the use of organic compounds as stabilizers (30). For the latter might be mentioned gum arabic, gelatin, sugar, glycerol, sodium citrate, saponin, barium arabinate, sodium protalbinate, and sodium lysalbinate.

Probably colloidal silver has received as much attention as has colloidal gold. Wool-fat has been employed as a stabilizing agent in preparing colloidal silver from organic solutions. Kohlschutter (31) reduced AgOH by means of hydrogen. Luppo-Cramer (32) obtained a series of beautifully colored silver sols by the reduction of silver nitrate

with hydroquinone in the presence of gelatin. Carey Lea (33) reduced $AgNO_3$ with ferrous citrate, dissolving the the deposit in water and reprecipitating with ammonium nitrate.

Svedberg (34) observed that a silver plate submerged in water or alcohol produced a silver colloid when illuminated by ultra-violet light or X-rays. According to Traube-Mengarini (35) a certain amount of silver colloid may be produced by boiling in water .

Nordenson (36) showed that silver is oxidized by both water and alcohol and is dissolved as AgOH or some other compound. This silver solution, Svedberg has shown, may be reduced by illumination, while Traube-Mengarini produced similar results with traces of reducing agents. The dissolution (or oxidation) is accelerated by light, especially ultra-violet light.

The method described in this paper depends upon the ability of aqueous extracts of seeds to reduce silver nitrate. A number of methods for preparing colloidal silver have already been mentioned. This is an addition to the list and is highly recommended because of its simplicity of manipulation. It should therefore be of more than passing interest to Plant Physiologists and Botanists as well as to Chemists.

The Method

One gram of timothy seeds is stirred into 100 cubic centimeters of distilled water and allowed to stand about an hour. The solution is filtered and two drops of

N/10 $AgNO_3$ added. If the solution is then permitted to stand in diffused light for an hour a dark brown color will appear, this being due to colloidal silver. The speed of the reaction may be increased by exposure to sunlight. However, too great exposure will precipitate the silver. If the aqueous extract is placed over a Bunsen burner and heated as soon as the $AgNO_3$ is added, the colloidal silver will form within a few minutes.

If the colloidal silver is not placed in the strong sun light the sol remains indefinitely stable. A solution in the laboratory at present has held up for over nine months.

The following seeds were later employed for reducing the silver, positive results being obtained in all cases except clover: pea, bean, tomato, clover, corn, wheat, buckwheat, grass, sunflower, lettuce and beet. Later investigation revealed that though there was a precipitate formed in the clover solution, the supernatant liquid was colored a faint brown like that of other sols.

In a subsequent experiment colloidal silver solutions were prepared from the following: corn, oats, wheat, rice, peas, beans, soy beans, cotton, beet, and gladiolus (corm). In this experiment 50 and 100 seeds were soaked in 100 cubic centimeters of water. These were all placed under the ultra-microscope and were found to exhibit Brownian movement. In the sol prepared from the oats the particles were so numerous that the field presented the appearance of a confused mass of seething particles.

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A platinum wire was placed on each side of the ultra-microscopic field and connected to storage batteries by means of a key. If the circuit were closed while the field was being observed the charge on the particles could be determined by their behavior in the electric field. All of the above samples possessed negative charges.

An outstanding feature of these colloidal solutions is the elaborate coloring. Colors varying from a dark amber to a rich rose or orchid were obtained. It is possible that different classes might be characterized by the color of their colloidal solutions. Corn, wheat, oats and rice all produced colloidal silver in a rose-colored suspension. Peas, beans, and soy beans were all characterized by a brown color. Other seeds were examined but could not be compared because no other closely related groups were included.

In an effort to locate the exact source of this reducing substance different parts of bean seeds were soaked in water and the solutions treated with $AgNO_3$. Beans were first soaked in distilled water over night. The next morning they were taken from the water, the seed coats removed, and the plumule and hypocotyl cut out of the cotyledons. These three parts (seed coat, cotyledons, and hypocotyl and plumule) were again soaked over night. On the following morning $AgNO_3$ was added to the extracts.

The intensity of the colloidal silver color was greatest with the seed coats, next with the cotyledons and least with the hypocotyl and plumule. In the last, the

color did not form until after several hours.

Shull (39) and Crocker (40) both hold that delayed germination is due to the inability of oxygen to reach the embryo. Is it possible that we have here a reducing substance in the seed coat that tends to decrease the amount of oxygen reaching the embryo during the period of dormancy?

The Nature of the Reducing Agent

About ten cubic centimeters of the aqueous extract of timothy seeds was enclosed within a collodion sack and lowered into a beaker of distilled water. Two or three drops of $AgNO_3$ were added to this distilled water and in a few days the extract within the collodion sack had assumed a brown color characteristic of the colloidal silver. This would indicate non-diffusibility of the part of the reducing agent.

A more concentrated solution of the timothy extract presents a dark brown color, indicative of tannin. In the presence of ferric chloride a faint blue-black precipitate is formed, the intensity of color resembling that produced by a .001% tannin solution when treated in the same way.

Following this a timothy extract was treated with lead acetate to remove the proteins. The excess lead was removed with H_2S . A few drops of $AgNO_3$ were added and a brown colloid, similar to the original, was formed. However, the colloid soon settled out.

The experiment was repeated, first adding enough

tannin to make it a .001% solution. The same brown colloid was formed, appearing permanent at first, but settling out after a longer interval. This filtrate did not reduce Fehling's solution.

The instability of this last colloid was no doubt largely due to the presence of acid formed by the method employed for removing the excess lead. Later sodium carbonate was used instead of hydrogen sulfide and the filtrate treated with $AgNO_3$. This time a colloid formed which was not quite the same color as the original. This colloid lasted longer than the others (about a week) but finally settled out like the rest.

Difficulty in identifying the reducing agent arises from the fact that many substances are extracted from the seeds by both water and alcohol. In some instances sugar has been found and in others it has not..

An alcoholic (90%) extract of corn meal has been found to reduce silver nitrate, producing a colloid identical in appearance with the original aqueous solution colloid. This silver sol thus far rivals the aqueous extract sol in stability.

In another experiment an alcoholic extract of corn meal was evaporated and the alcohol replaced with water as the alcohol evaporated. The zein was precipitated in this process. This impure zein was dissolved in alcohol (ethyl) and $AgNO_3$ added to the solution. A silver sol, very similar

to the original was formed. After a period of a week this sol became cloudy and appeared to be precipitating.

From the above results it seems possible that both sugar and zein would be extracted by the alcohol. When the alcohol is replaced by water the sugar would go into solution, leaving the zein to be precipitated. Hence it might be suspected that the silver can be reduced by either the alcohol- or water-soluble proteins and the sol stabilized by sugar or vice versa.

An aqueous extract of corn meal was made after the meal had first been extracted with 90 % alcohol. This aqueous extract reduced Fehling's solution when tested for sugars. There were also proteins present, as was evidenced by the precipitate formed in clarifying the sugar solution. A colloidal silver solution was produced by this aqueous extract but the sol was unstable.

The zein thus far mentioned in this paper has been designated as impure zein. It was prepared by extracting corn meal with alcohol, evaporating off the alcohol, replacing the alcohol with water as the alcohol evaporated, and collecting the zein as it precipitated.

A little later some pure zein was prepared. An alcoholic extract of corn meal was made, the zein salted out with dilute NaCl, and the NaCl removed by dialysis. It is possible that some of the carotinoid pigments were brought down with the zein but we shall distinguish between the two by calling the last one "pure zein".

A stable colloid has been prepared by dissolving the "pure zein" in alcohol and adding $AgNO_3$. With the impure zein the colloid was not stable unless a small amount of arabinose was added. These last two colloids have been called stable because they have held up longer than any of the other artificially prepared sols. As a matter of fact, their appearance at the end of two weeks would indicate that though they are stable, they probably will not last as long as the aqueous and alcoholic extract sols.

Summary

(1) The literature dealing with the preparation of colloidal metals by condensation has been briefly reviewed.

(2) A simple method has been described for preparing colloidal silver from aqueous extracts of seeds.

(3) Colloidal silver may also be prepared from alcoholic extracts of seeds, alcoholic solution of pure zein, and an alcoholic solution of impure zein plus arabinose. The stability of the last two is not guaranteed.

(4) The reducing agents are not as yet known but it seems probable that they might be alcohol- and water-soluble proteins with sugar as the stabilizer.

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