The Selective Permeability of the Coverings of the Seeds of Hordeum vulgare.

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The seeds of the variety of barley known as *Hordeum vulgare* var. *cœrulescens* owe their colour to the presence of a blue pigment in the aleurone cells; this pigment, like litmus, is turned red by acids. Such seeds, when immersed in a dilute solution of sulphuric acid, if their coverings are damaged, soon turn pink in colour, which is a proof that acid diffuses into the endosperm; sound seeds, on the other hand, although they imbibe water freely from the solution, becoming soft and swollen, retain their colour, showing that the covering has the property of resisting the passage of the acid, whilst it allows water to diffuse freely into the interior of the grain. So much is this the case that a dilute solution of sulphuric acid may be concentrated by steeping barley in it. Thus in an experiment with a solution containing 4·9 grammes of acid per 100 c.c. it was found that the concentration of the acid was increased to 7·6 grammes per 100 c.c. In another case, in which the weight of water absorbed was ascertained, it was observed that the concentration effected was in direct proportion to the amount of water absorbed by the seeds.

Having made the discovery of so remarkable a "semi-permeable" membrane, I have endeavoured to ascertain its behaviour towards substances generally. In my earlier experiments, of which an account has been given elsewhere,* it was found that sulphuric acid could not penetrate into the grain, not only from volume normal solutions, but also from solutions containing 9, 18, or even 36 grammes of acid per 100 c.c. In the case of the seeds immersed in the strongest acid, however, the interior remained dry, presumably because the power of the seed contents of imbibing water was insufficient to overcome the osmotic pressure of the liquid.

The vitality of the embryos was not destroyed by steeping the seeds in the acid solutions; when placed under suitable conditions they all germinated.†

When the blue seeds were immersed in a volume normal solution of

* 'Annals of Botany,' vol. 21, p. 79, 1907.

† Recent observations show that the barley corn displays a most remarkable power of withstanding the action of sulphuric acid. A number of blue corns, *i.e.*, those containing the neutral indicator for acid, were steeped in a volume normal solution of sulphuric acid during 48 hours, those corns which showed traces of red after this treatment being rejected

hydrogen chloride, the colour remained unaltered, showing that there is no diffusion of acid into the grain.

Solutions of caustic soda containing 1 per cent. or more of alkali disintegrate the seed covering; this resists the action of the alkali, however, if the liquid contain only ½ per cent., and after steeping seeds during several days in the solution, although water diffuses into the grain, no alkali enters.

Salts such as cupric sulphate, ferrous sulphate, potassium chromate, and silver nitrate were all found to be impenetrant substances.

Up to this point, it appeared that the covering of the seeds was a perfect "semi-permeable" membrane. Using iodine dissolved in a solution of potassium iodide, however, observations were made which indicated that the membrane possessed the power of selection—iodine was found to pass slowly into the seed until after several days it permeated the whole of the starchy endosperm, staining it a deep blue colour. That this result was not a consequence of the destruction of the membrane was proved by steeping seeds thus impregnated with iodine in a solution of sodium thiosulphate. So long as the seed coverings remained intact the iodine was unaffected, but when the coverings were ruptured the thiosulphate diffused rapidly into the seeds decolorising the iodine.

At this point my earlier studies were directed to an investigation of the nature and position of the particular covering of the seed of *H. vulgare,* which acted as the "semi-permeable" membrane. The experiments already described demonstrate that the embryo and endosperm of the seeds are enclosed within an envelope through which water and iodine diffuse readily, but through which salts and strong acids do not diffuse. As there appeared to be no recognised instance in the vegetable kingdom of a membrane other than one composed of living protoplasm possessing marked "semi-permeable" properties, it was obviously desirable to ascertain if the selective permeability of the seed-coverings were a function of the living tissue. From the

as faulty. Subsequently, the corns which remained blue were steeped continuously in the acid, and observed from time to time, with the following results :—

Time of steeping.	Percentage of corns remaining blue.
3 days	100
5 ,,	99
7 ,,	95
10 ,,	89
14 ,,	74
19 ,,	25
24 ,,	1

first this appeared to be very improbable, as the property was exhibited by the seeds in the presence of highly toxic acids and salts, which could hardly fail to arrest its vital activity if brought into contact with protoplasm. Experiments made with seeds which have been immersed in boiling water during varying periods extending to one hour have afforded conclusive evidence that the semi-permeability of the seed-coverings is not a function of living protoplasm.

Histological study of the seed-coverings indicates that their selective power is confined to the testa, and probably to that portion which is derived from the epidermis of the nucellus during the development of the seed.

It appeared to be very desirable that the coverings should be removed and their behaviour studied apart from that of the seed contents. Although this has been attempted, the many experimental difficulties met with have not been satisfactorily overcome, and hitherto no other means of investigating their activity has been found than the somewhat unsatisfactory one of experimenting with whole seeds.

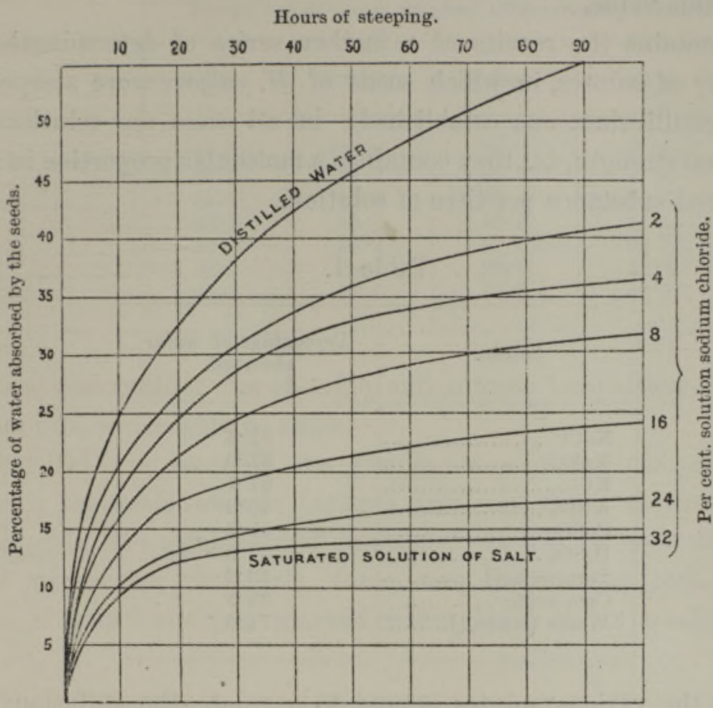
Behaviour of the Seed of H. vulgare *as a Semi-permeable System possessing a strong affinity for Water.*

When sound seeds of *H. vulgare* are immersed in an aqueous solution containing a substance which cannot pass through the outer semi-permeable membrane, and water is absorbed, presumably the seed contents enter into competition with the solute for the water. From this point of view, it appeared to be important to ascertain to what extent water would be absorbed from various solutions, and to compare the amounts with that absorbed when the seeds were placed in water alone.

The method adopted in all the experiments to be referred to was as follows:—A known weight of selected air-dried seeds, usually about 5 grammes, having been steeped in the solution under examination during some desired period, the seeds were separated from the liquid by means of a small wire-gauze strainer. After draining for a few minutes they were placed between the folds of a soft, dry cloth and were rubbed gently to remove as much of the adherent liquid as possible; they were then weighed. It is obvious that such a method cannot afford absolute values; but experience shows that concordant results may be arrived at without difficulty if all operations in connection with draining and drying the seed are carried out in as constant a manner as possible. The water absorbed is expressed as a percentage weight calculated on the original dry weight of the seeds.

The results of a series of observations with solutions of common salt con-

taining from 2 to 32 grammes of salt per 100 grammes of solution are recorded graphically in the accompanying diagram.



It will be noticed that the amount of water absorbed by the seeds when equilibrium is established is less the more concentrated the solution, as it varies from about 14 per cent. in the case of a saturated solution to about 41 per cent. in the case of a solution containing only 2 per cent. of salt; this latter amount is much below that absorbed when the seed is steeped in water alone (over 70 per cent.).

It should be pointed out that the determinations on which the curves are based are affected by an unavoidable error due to the manner in which the values are arrived at. Some allowance should be made for the amount of water absorbed by the outer covering of the seeds. It is not possible to evaluate this amount very closely; apparently, however, it may be taken as equal to about 8 per cent. of the original weight of the dry seed. Making the extreme assumption that the amount absorbed by the outer covering is independent of the concentration of the solution, the quantity absorbed by the starchy contents of the seed from a saturated solution of salt is only about (14 − 8 =) 6 per cent. calculated on the weight of the dry seed. It appears, therefore, that the power of the seed contents of attracting water from a saturated solution of salt exceeds the osmotic attraction of the latter to only a slight extent; as the "osmotic pressure" of a saturated solution

of sodium chloride is about 125 atmospheres, the power with which water is absorbed by the seed contents probably corresponds to a pressure somewhat in excess of this value.

Table I contains the results of a further series of determinations, made with a variety of solutes, in which seeds of *H. vulgare* were steeped in the liquid until equilibrium was established; in all cases the solutions were of volume normal strength, *i.e.*, they contained a molecular proportion in grammes of the dissolved substance per litre of solution.

Table I.

Solute.	Percentage of water absorbed.
NaCl	37·4
$NaNO_3$	37·9
KCl	37·1
KNO_3	40·5
$CuSO_4$	41·7
H_2SO_4	37·8
Tartaric acid	42·2
Cane-sugar	39·3
Water (control)	74·3

Although the various solutes appear to regulate the diffusion of water into the seeds in a very similar manner, minor differences are observable; thus, contrasting sodium chloride with potassium nitrate, there is an excess of 3 per cent. in the amount of water absorbed from the solution of the latter. This small excess was at first regarded as an experimental error, the method being open to suspicion when small differences are concerned; but as all experiments made subsequently with the same salts have consistently afforded similar results, there can be little doubt that the small departure is real. Small differences are also noticeable between the values for cupric sulphate and tartaric acid in comparison with that afforded by sodium chloride.

As the amount of water present in a *volume normal* solution varies from substance to substance, experiments were now made with *weight normal* solutions equivalent in strength, prepared by dissolving the solute always in the proportion of 1 gramme molecular proportion to 1000 grammes (55·5 molecular proportions) of water. The results obtained are recorded in Table II, the values in the last column being those observed when equilibrium was established. Although they are in close general agreement with those obtained on using volume normal solutions, small specific differences between the salts become apparent, the potassium salt being less

Table II.

Solute.	Percentage of water absorbed after steeping the seeds during—				
	2 days.	4 days.	6 days.	8 days.	11 days.
KCl	31 ·2	36 ·5	36 ·9	37 ·3	38 ·4
NaCl	30 ·5	34 ·2	35 ·7	37 ·3	37 ·2
NH_4Cl	31 ·7	34 ·6	36 ·4	36 ·6	37 ·4
KNO_3	34 ·1	38 ·7	40 ·5	41 ·1	41 ·6
$NaNO_3$	32 ·5	36 ·3	37 ·5	38 ·7	38 ·5
NH_4NO_3	32 ·3	36 ·1	38 ·3	38 ·4	38 ·9
Water (control)	43 ·1	55 ·6	64 ·1	68 ·3	70 ·0

active in the case both of the chloride and nitrate than either the sodium or ammonium salt, which behave alike.

In Table III are recorded the results of a direct comparison of the behaviour of the seeds towards dextrose and cane sugar in comparison with sodium chloride, in weight normal solutions. It will be seen that, although distinctly less active than salt, these two substances both inhibit the absorption of water to a very marked extent, being about equally effective.

Table III.

Solute.	Percentage of water absorbed in—				
	2 days.	4 days.	6 days.	7 days.	11 days.
Cane-sugar	29 ·5	34 ·3	36 ·2	36 ·9	38 ·4
Dextrose	30 ·2	35 ·8	38 ·1	38 ·3	39 ·8
NaCl	28 ·1	31 ·9	34 ·2	34 ·4	35 ·5

Selective Permeability of the Seed-coverings of H. vulgare.

Mercuric Salts.—When barley seeds are steeped in a 3-per-cent. solution of mercuric chloride in water, the salt may be detected within the seed covering after a few hours; after two or three days it is usually found diffused throughout the whole of the interior portions of the seeds.

At first it seemed possible that the passage of the salt into the seed ought not to be regarded as proof of a selective property of the seed-covering, but perhaps merely as an indication that the action of the salt destroyed their semi-permeable character. Experiment, however, has proved conclusively that this is not the case. When seeds which have been steeped

in a solution of mercuric chloride during several days and then dried were steeped in a normal solution of sulphuric acid, it was found that the coverings still retained their original power of resisting the diffusion of sulphuric acid while permitting the diffusion of water into the seed.

Still more conclusive evidence of the possession by the seed-coverings of a differentiating power was furnished by an experiment in which seeds were steeped in a mixture of half a volume of a saturated solution of mercuric chloride with half a volume of normal sulphuric acid. After three days' steeping in this solution the mercuric salt was found to be diffused throughout the contents of the seed; even after five days' steeping, however, no trace of sulphuric acid could be found within the seed-coverings. The seeds of *H. vulgare* therefore possess the very remarkable property of absorbing mercuric chloride and rejecting sulphuric acid when steeped in a solution in which both are present. The exhibition of this property by the seeds appears to be of very special interest from a physiological point of view.

To ascertain their behaviour towards mercury salts generally, seeds were steeped in solutions of mercuric chloride, cyanide, nitrate and sulphate of equimolecular strength. The cyanide diffused as readily as the chloride into the seeds, but after several days no trace of mercuric salt could be recognised in those placed in the solutions of nitrate and sulphate. Moreover, the amount of water absorbed by seeds from solutions of mercuric chloride and cyanide did not differ from that taken up from water alone.

It should be noticed in passing that chloride and cyanide of mercury—which diffuse through the seed-coverings—are commonly regarded as but very slightly dissociated in aqueous solution, whilst mercuric sulphate and nitrate—which cannot penetrate the membrane—are salts which are supposed to be freely dissociated.

Cadmium Salts.—On steeping seeds of *H. vulgare* in volume normal solutions of cadmium iodide, chloride, and sulphate until equilibrium was established, the following results were obtained:—

Table IV.

Solute.	Percentage of water absorbed.
CdI_2	54·2
$CdCl_2$	46·3
$CdSO_4$	46·0
NaCl (control)	39·8

It will be noticed that the gain in weight in presence of the iodide is markedly greater than in presence of the chloride or sulphate, but that none of the salts has so considerable an effect as sodium chloride.

In the case of the seeds steeped in the solution of cadmium iodide—a salt which is supposed to be very slightly dissociated when dissolved in water—small quantities of the salt diffused through the coverings; cadmium was not detected, however, in the seeds which had been steeped in solutions of either the chloride or the sulphate, which are supposed to undergo dissociation to a somewhat limited extent, although to a greater extent than the iodide.

Acetic and other Weak Acids.—On searching for compounds capable of passing through the seed-coverings, when it was found that acetic acid possesses the property, it at first seemed probable that the acid might be capable of destroying the semi-permeable layer of the seed. Seeds were therefore steeped in solutions containing both acetic and sulphuric acids or acetic acid and cupric sulphate; in both cases only acetic acid and water diffused through the coverings.

Water also passes freely together with the acid into the seeds. The results of experiments with a volume normal solution of the acetic acid are recorded in Table V; it will be noticed that the acid has but a slight influence in diminishing the amount of water which is absorbed when equilibrium is established.

Table V.

Solute.	Percentage of water absorbed.
Acetic acid	73·8
Water (control)	78·2
Sodium acetate (control)	39·8

On examining the behaviour of the seeds towards organic acids other than acetic, it was found that formic, propionic and butyric acids also enter the seed system, and that they affect the introduction of water much as acetic acid does.

Glycollic acid, although excluded during about 48 hours, subsequently diffuses slowly into the seed. Lactic acid did not enter most of the grains until after the lapse of 72 to 96 hours. The amount of water absorbed by the seed is diminished more by these acids than by acetic acid, thus:—

Table VI.

Solute.	Percentage of water absorbed during—					
	2 days.	4 days.	7 days.	9 days.	10 days.	11 days.
Glycollic acid	33 ·5	43 ·9	51 ·5	57 ·6	59 ·5	63 ·4
NaCl	28 ·2	31 ·9	34 ·1	35 ·2	35 ·7	36 ·0
Water	40 ·1	53 ·6	63 ·8	68 ·6	69 ·0	70 ·3
					11 days.	13 days.
Lactic acid	37 ·4	45 ·1	52 ·8	55 ·1	58 ·1	61 ·4
NaCl	30 ·9	34 ·4	35 ·8	36 ·8	36 ·5	

Trichloracetic Acid.—This acid was chosen on account of its similarity in configuration to acetic acid, from which it is distinguished, however, by being a strong electrolyte, acetic and the other acids and the salts which diffuse through the seed-coverings being all weak electrolytes.

On immersing seeds in a solution containing 5 per cent. of the acid, it was found to enter them very rapidly, so much so that after 48 hours they were saturated with it. This result was clearly not due to any destructive action of the acid on the seed-coverings, as when seeds saturated with a solution of trichloracetic acid were immersed in a solution of sodium bicarbonate, the acid within the seeds remained unaffected even after the lapse of 10 days. In a control experiment, seeds impregnated with acid, of which the coverings were intentionally damaged, were placed in a solution of the bicarbonate; this soon entered the seed and in a few hours neutralised the acid. Trichloracetic acid is the only strong electrolyte which has been found to possess the property of diffusing into the seed system.

Ammonia.—The membrane is more or less injured by exposure of the seeds in solutions of ammonia of weight normal strength, as acid penetrates into the corns after they have been steeped in such a solution. On the other hand, when corns which had been steeped in one-half or one-quarter normal solutions of ammonia were dried and then exposed in a normal solution of sulphuric acid during 48 hours, no acid was found to enter. The velocity with which water is absorbed from solutions of ammonia is remarkable, as shown by the results recorded in Table VII.

The ammonia passes into the corns with the water; on the other hand, when the corns impregnated with ammonia are placed in a normal solution of sulphuric acid, after 24 hours they are no longer alkaline internally, the ammonia having passed out in the reverse direction.

Table VII.

	Percentage of water absorbed.			
	2 days.	4 days.	6 days.	8 days.
Ammonia—				
Normal solution	53·5	70·1	74·8	77·2
½ " "	53·9	68·5	73·5	74·2
¼ " "	51·5	65·9	72·3	72·2
Sodium chloride (control)—				
Normal solution	29·4	32·2	33·3	34·6
½ " "	32·2	37·1	39·4	40·4
¼ " "	35·1	41·7	45·7	48·4
Water	43·1	55·6	64·1	68·3

Non-electrolytes.—As previously pointed out, cane-sugar and dextrose resemble the electrolyte sodium chloride in their power of diminishing the extent to which water is absorbed by the seed system, and in being unable to penetrate the seed-covering.

Experiments made with a number of non-electrolytes of much lower molecular weight than the sugars show the behaviour of these to be comparable with that of weak electrolytes.

The following table contains the results of experiments with volume normal solutions of ethyl alcohol, aldehyde, acetone, and ethylic acetate. For purposes of comparison, the results obtained at the same time with water and with volume normal solutions of acetic acid (representing a freely diffusible solute) and of sodium acetate (representing a non-diffusible solute) are given :—

Table VIII.

Solute.	Percentage of water absorbed.
Ethyl alcohol	74·0
Aldehyde	70·6
Acetone	72·7
Ethylic acetate	73·1
Acetic acid (control)	73·8
Water (control)	78·2
Sodium acetate (control)	39·8

The results indicate that water is absorbed by the seed-system from solutions of alcohol, aldehyde, acetone, and ethylic acetate approximately as it is absorbed from that of acetic acid, or when in contact with water alone.

Very similar results were obtained on using weight normal solutions.

Experiments in which seeds were placed in contact with alcohol, aldehyde, acetone, and ethylic acetate in the anhydrous condition have shown that these

Table IX.

Solute.	Percentage of water absorbed during—				
	2 days.	4 days.	7 days.	9 days.	11 days.
Ethyl alcohol............	43·4	54·9	66·9	68·7	69·6
Ethylic acetate	63·9	70·7	72·8	72·1	71·8
Acetic acid...............	53·3	67·6	68·3	68·5	68·0
Water (control).........	45·0	55·6	65·5	68·9	70·5
NaCl (control)	30·9	34·4	35·8	36·8	36·5

substances do not diffuse through the seed-coverings in the absence of water, although they all diffuse readily into the interior of the grain from their aqueous solutions.

It is also interesting to note the manner in which the velocity with which the different solutions are absorbed varies. The solution of ethyl alcohol enters comparatively slowly, at about the same rate as pure water; that of acetic acid enters more rapidly; whilst the rate of entry of the solution of ethylic acetate is markedly the most rapid of the three. Nevertheless, despite the differences of velocity, equilibrium is established between the seeds and the three solutions at approximately the same point.

A further series of observations with solutions of non-electrolytes are recorded in the following table:—

Table X.

Solute.	Percentage of water absorbed.
Glycerol	41·5
Glycine	41·8
Urea	45·5
Ethylene glycol.....................	52·7
Sodium chloride (control)	36·5
Water (control)....................	20·5

The results obtained with glycerol and glycine resemble those afforded by cane-sugar and dextrose (see Table III), but differ very markedly from those obtained with such compounds as alcohol and acetic acid. The behaviour of glycine or amino-acetic acid is particularly interesting, as this compound differs to so slight an extent in constitution from acetic acid. Urea and ethylene glycol have less influence than either glycerol or glycine; glycol, however, although it differs to so slight an extent from alcohol in constitution, is far more effective in preventing the entry of water.

Summary of Conclusions.—The investigation of the selective properties of the semi-permeable seed-coverings of *H. vulgare* described in this paper should be regarded as pioneer work only; much further study is required in order to explain the varying actions of the seeds in the presence of different solutes in aqueous solution.

At present, the general trend of the evidence tends to show that solutions of the solutes which diffuse readily through the seed-coverings differ in some essential manner from solutions of non-diffusible solutes, although the nature of the difference remains unexplained. The results of some of the earlier experiments described above appear to support the view that the property of diffusion is intimately associated with a low degree of "ionisation" of the solute; yet the conspicuous instance which has been noticed of the ready diffusibility of trichloracetic acid, a highly "ionised" acid, tends to show that such correlation, if it exist at all, is not an intimate one. Further, the view does not appear to be favoured by those experiments which have demonstrated that certain non-electrolytes, such as ethyl alcohol, are readily diffusible, whilst others, such as glycerol, are non-diffusible.

In connection with the same question, it seemed possible that differences in the surface tension of solutions of diffusible and non-diffusible solutes might perhaps be associated in some way with the different behaviour of the two classes of solutions towards the seed-coverings; but it appears from a study of the surface tensions of the two classes of solutions that there is no such intimate connection between them. Neither can any indication be found that viscosity is associated with the manner in which diffusible and non-diffusible solutes behave differently towards the seed-coverings.

The only explanation of the observed difference in activity of the two classes of solutions which at present suggests itself as a working hypothesis is, that some unrecognised 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 behaviour 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 solutions 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.