the spread of cotton rust in the Rio Grande valley of Texas.

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SPECIAL ARTICLES

THE EFFECTS OF ACIDS AND SALTS ON "BIO-COLLOIDS"

MIXTURES of agar with gelatine, albumen, protein, urea or amino-acids in which the agar forms seventy-five per cent. or more of the whole, show a similarity of imbibitional behavior to that of sections of plants and hence for convenience in the present studies may be termed "*bio-colloids.*" The results of a series of tests with a wide variety of nitrogenous substances from urea to albumen were in general agreement to the effect that such substances mixed in proportions of one to ten, more or less, with agar, when made into thin dried plates, swelled enormously; 2,000 to 3,000 per cent. in distilled water, one half to one tenth this amount in hundredth-molar hydrochloric acid, and more or less in hundredth-molar sodium hydrate.[1]

An extension of the tests of the effects of nitrogenous substances upon the swelling of the amorphous carbohydrates was made to include a mixture of agar and peptone the swelling measurements of which were as follows:

AGAR 90—PEPTONE 10

Water	HCl *M*/100		NaOH *M*/100
3166.6%	500%	(20 hours)	633%
———	566.6	(20 hours)	800
———	633.3	(48 hours)	1,666.6

The chief feature of interest in these results is the uniform swelling in alkali in excess of that in hydrochloric acid, in a manner slightly different to that of similar mixtures in which other nitrogenous substances were used.

[1] See "Growth and Imbibition" presented before the American Philosophical Society, April, 1917, and now in press in the *Transactions* of the society; also "The Behavior of Certain Gels Useful in the Interpretation of the Action of Plants," SCIENCE, 43, p. 484, 1917.

The chief purpose of the entire series of studies has been to ascertain what conditions of growth and development might be identical with the factors affecting imbibition. The fact that plant protoplasts usually consist of a large proportion of carbohydrate gels with a smaller proportion of nitrogenous material has already been discussed. The resulting colloidal mixture may be acidified as a result of certain respiratory processes, or this acid may be broken up as fast as formed, in which case the protoplast might be in a deacidified or neutral condition and from this might vary to alkaline under conditions which we are not yet ready to describe. Acidification and deacidification of the cell may take place at a rapid rate and be complete within a short time, according to the bulk of the cell-mass, temperature and other conditions.

Hydrochloric acid had been used in nearly all of the earlier work for acidification of colloids, since most of the known facts as to the swelling of gels are referable to it. The acids of the plant are organic, and a modification of the technique to heighten the similarity between the experiments and the action of the plant was to substitute citric for hydrochloric acid in the series.

Preliminary to this substitution, series of swellings were carried out to test the relative action of the two acids, with the following results from dried plates of mixtures of 90 parts agar and 10 parts bean protein:

Hydrochloric Acid *M*/100	Citric Acid *M*/100	Sodium Hydrate *M*/100
541.6%	916.6%	916.6%
	875	
	875	
300	402	400

The effect of this organic acid in this initial series of tests was to produce an imbibitional swelling fairly equivalent to that of sodium hydrate and to cause such colloidal mixtures to take up more water than in hydrochloric acid. An extended series of measurements will be necessary before any serious conclusion can be formulated, however.

Another set of factors arising from the presence and concentration of salts is next to

be considered. Certain of these compounds are invariably present, although in varying concentration, and any attempt to apply studies of imbibition to swelling and growth problems must take into consideration the fact that the various reactions due to the presence or proportion of nitrogenous compounds, alkalinity or acidity, invariably ensue in saline solutions, attenuated as they may be in young protoplasts. Tests were therefore planned to determine the action of the common bases and acids on bio-colloids.

Agar which has been used to represent the carbohydrate constituent of living matter gave the following results when dried plates .28 mm. in thickness were tested:

Water	Potassium Nitrate		
	M/100	*M*/50	*M*/10
2,325%.....	1,535.7%	910.7%	607.1%
	Calcium Nitrate		
	785.7	——	500

The amount of swelling as compared with distilled water was decreased by both salts and the inhibiting action increased with the concentration.

A mixture of 90 parts agar and 10 parts of glycocoll gave the following swellings:

Water	Potassium Nitrate		
	M/100	*M*/50	*M*/10
3,266.6%.....	1,800%	1,733.3%	1,333.3%
	Potassium Chloride		
	1,733.3	1,666.6	900
	Calcium Nitrate		
	1,333.3	1,200	800

From which may be seen that an inhibiting effect on imbibition in the bio-colloid similar to that of agar was exerted by these salts, the effect increasing with the concentration and the least swelling taking place in the calcium compounds.

A mixture of 90 parts agar and 10 parts of peptone gave the following swelling measurement.

Water	Potassium Chloride *M*/100	Potassium Chloride, Hydrochloric Acid *M*/100
2076%	1230.8%	500%

Water	Potassium Nitrate		
	M/100	*M*/50	*M*/10
3,166.6%.....	1,600%	1,300%	866.6%
	Calcium Nitrate		
	1,133.3	1,133.3	733.3
	Potassium Chloride		
	1,266.6	——	1,000.0

The lessening or inhibitory effect is seen to increase with the concentration, and less swelling takes place in equivalent calcium solutions than in potassium. The irregularities, however, suggest that peptone mixtures present some special characters which will need further analysis.

Dried plates of a mixture of 90 parts agar and 10 parts urea gave the following swelling measurements:

Water	Potassium Nitrate		
	M/100	*M*/50	*M*/10
2933.3%.....	1533.3%	1233.3%	766.6%
2933.3	1133.3	813.3	700
	Calcium Nitrate		
	813.3	813.3	500

These results are in general accord with those obtained from other nitrogenous mixtures.

A mixture consisting of 10 parts of gelatine and one part of mucilage from *Opuntia* might be considered as equivalent to the colloids consisting of 90 parts gelatine and 10 parts agar, and gave the following swelling measurements.

Water	Potassium Nitrate		
	M/100	*M*/50	*M*/10
589.4%.....	485.5%	455.3%	698.2%
	Potassium Chloride		
	473.2	473.2	401.9
	Calcium Chloride.		
	473.2	.....	348.2

The swelling increases within the range of concentration of potassium nitrate used, and appears to decrease slightly within similar concentrations of potassium chloride, and is checked to a greater extent by calcium chloride, although the last named solution would have a slightly alkaline reaction due to the hydrolysis of the salt.

The effect of salts alone on the bio-colloid in which gelatine forms the nitrogenous constituent is shown by the following measurements of the swelling of a series of dried plates of 90 parts agar and 10 of gelatine, .22 mm. in thickness:

Water	Potassium Nitrate		
	M/100	*M*/50	*M*/10
1,136.4%.....	940.9%	772.7%	613.7%
	Calcium Nitrate		
1,454.5%.....	840.9	704.5	409.1
	Potassium Chloride		
	M/100	*M*/50	*M*/10
	1,000	772.8	590.9
	Calcium Chloride		
	704.5	545.4	386.4
	Sodium Chloride		
	939 (average of 3 tests)		

The next step to be taken was one in which the effect of the universally present salts were tested in various concentrations in connection with conditions of acidity and of deacidity.

As an example of such tests the results obtained by a study of the action of dried plates of a mixture of 90 parts agar and 10 parts bean protein are given below:

Calcium Chloride *M*/100	Calcium Chloride Hydrochloric Acid *M*/100
769.2	538.5

It is apparent from these results that acidity decreases the amount of imbibition in the presence of the salts tested.

A few tests made to determine the limits of imbibition in concentrated solutions revealed the fact that dried plates of 90 parts agar and 10 parts of bean protein swelled 576.9 per cent. in a saturated solution of potassium nitrate which has an osmotic coefficient of about 60 atmospheres. The same material swelled 730.9 per cent. in a solution of 50 g. of calcium nitrate in 100 cc. of water (2-molar solution) which has an osmotic coefficient of about 44 atmospheres. A swelling of 100 per cent. was shown in a 3-molar solution of calcium chloride and if hundredth molar hydrochloric acid was added the swelling was increased to as much as 200 per cent. These facts illustrate very forcibly the possibilities of imbibitional absorption against osmotic action. The significance of such action in parasitism and nutritive couples has been discussed elsewhere.[2]

All tests in which the samples of colloid are presented to the action of the reagent in a neutral and dried condition are of course widely different in hydratation conditions from those prevalent in the protoplast. The colloids of the living material are continuously subject to interaction and to modifications resulting from the action of salts, acids, alkalies and their combinations.

A few tests in which plates of bio-colloid swelling from the action of one solution are subjected to another have already been described. The possibilities presented, however, are such as to justify the minutest examination.

In one series dried plates of 90 parts agar and 10 parts bean protein were first subjected to the action of alkali, to hydrochloric acid and to citric acid separately for eighteen hours, at the end of which time their full imbibitional capacity had been reached under the separate influence of each of these reagents. The solutions were then pipetted off and a second reagent introduced. The initial and the secondary action are indicated below.

First Swelling

Hydrochloric Acid *M*/100	Citric Acid *M*/100	Sodium Hydrate *M*/100
300%	402%	400%

[2] See MacDougal, D. T., "The Beginning and Physical Basis of Parasitism," *Plant World*, 1917 in press.

A number of tests were made in which the same bio-colloid was successively subjected to a series of reagents with exposures of two hours or more to each one in succession as follows:

Sodium Hydrate $M/100$	Sodium Hydrate $M/100$	Hydrochloric Acid $M/100$
360	300	Slight and irregular shrinkage.

Plates of agar 90 parts gelatine 10 parts, .07 mm. in thickness swelled 1,143 per cent. in 45 minutes in distilled water, then 213 per cent. in $\frac{HCl}{KCl}\frac{M}{100}$ in 2 hours, then 430 per cent. in KCl(M/100) in the next 4 hours, after which it stood in the acidified potassium chloride solution without measurable change for 11 hours. The replacement of this solution by a hundredth molar sodium-hydrate solution was followed by an increased imbibition equivalent to 643 per cent. of the original plate in two hours, at the end of which period it had swelled altogether about 2,400 per cent. of its original thickness.

A similar plate swelled initially 3,357 per cent. in 14 hours in water, then shrank about 300 per cent. in a hundredth molar acidified potassium chloride solution in 11 hours, after which it swelled about the same amount in hundredth molar hydrate.

Some very striking results were obtained by plates .12 mm. thick of 90 parts agar and 10 parts bean protein. A trio of samples swelled 1,416.5 per cent. in 4 hours in distilled water, then shrank 208 per cent. in hundredth molar acidified potassium chloride in 3.5 hours, then swelled 643 per cent. in hundred molar sodium hydrate in 13 hours and 1,250 per cent. in distilled water in 14 hours. At the end of this time a total increase of about a hundred per cent. in hundredth molar hydrochloric acid took place. A second trio of same material swelled about 400 per cent. in less than an hour in water, then 200 per cent. in 3 hours in hundredth molar acidified potassium chloride solution, then 750 per cent. in 3.5 hours in hundredth molar sodium hydrate, 1,583 per cent. in water in 10 hours. After this total imbibition of about 2,500 per cent. had been reached immersion in hundredth molar acidified potassium chloride for 3 hours produced a dehydration of only 167 per cent., not all of which was regained when the acidified salt solution was replaced with water.

These two series serve to illustrate changes in imbibition capacity which might take place in the protoplast. It would be highly unwise to generalize upon the basis of the meager results available, yet the records described suggest certain reasonable assumptions. Among those may be included the inference that after a plate of bio-colloid is in a swelling stage the addition of an acidified salt solution checks the rate of swelling if the total amount is still below that possible in the solution. If the swelling is already beyond the total possible in the acidified salt solution some dehydration occurs, but by no means enough to reduce the swelling to the acidified salt total. Dehydration effects from hydrochloric or citric acid were very slight. The application of alkalies in advanced stages of swelling after acidified salt solutions seemed to increase swelling beyond the total possible in a simple immersion in alkali.

Analyses of modifications of growth rates must therefore take into account not the simple total effect of any solution upon the colloids of the enlarging protoplast, but upon these bodies as already modified by previously acting solutions.

The chief interest in all of the experimentation on imbibition described in this and in previous papers has been directed to various effects simulating growth by acids, alkalies, salts and combinations upon bio-colloids as illustrated by the mixtures described. The differential action which might ensue from the addition or subtraction of a nitrogenous compound from the carbohydrate body of protoplasts in special tracts, changing the imbibition capacity of chromosomes, of spindles or cell plates, etc., may well play an important part in the mechanics of mitosis and cell division.

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