SUMMARY

The microbiological method of Snell & Strong and the fluorometric method of Najjar for the assay of riboflavin have been improved and modified with particular reference to cereals and cereal products. Agreement between the results obtained by the two methods is good, and figures are presented covering a wide range of such products.

The microbiological method is preferred as it is more rapid than the fluorometric, and requires less material and no specialized apparatus.

We are greatly indebted to Miss Marjory Stephenson for much helpful advice and criticism.

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The Effect of Salts on the Formation of Protein Complexes during Heat Denaturation

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The separation of floccules from solutions of proteins denatured by heat is the final and most obvious stage in a process of aggregation. The factors controlling flocculation have been studied in detail by many workers, but little attention has been given to their effects on earlier stages of aggregation. If the heating is insufficient, or other conditions are unsuitable to cause flocculation, stable solutions of heat-changed proteins are obtained. When solutions containing more than one protein are heated in such conditions, aggregation may take place between particles of different proteins, when stable solutions of protein complexes are produced. Such complexes may have properties different from those of either the unheated mixture of proteins or the individual proteins heated separately. For example, such complexes are formed when serum albumin and globulin are heated together; these have been detected by electrophoresis [Van der Scheer, Wyckoff & Clarke, 1941], as well as by changes in the ease of sedimentation during centrifugation, and by changes in precipitability with salts [Kleczkowski, 1941*a*].

Antibodies and protein-antigens can combine to form complexes with other proteins simultaneously undergoing denaturation by heat. Van der Scheer *et al.* [1941] and Krejci, de Spain Smith & Dietz [1941] showed that when antisera are heated, antibodies are found in products with changed electrophoretic mobility, and Kleczkowski [1941*b*] and Bawden & Kleczkowski [1941, 1942] have described changes in the serological behaviour of both antibodies and protein-antigens after heating with unspecific serum proteins. These changes are most striking when 'O'-type antigens, giving granular precipitation with antisera, or their antibodies, are heated in the presence of serum albumin. The complexes formed by such antigens are no longer precipitated by antibodies, although they still combine with them to inhibit the precipitation of any unheated antigens added subsequently. Similarly, the complexes formed by antibodies still combine with the antigens but are unable to precipitate them.

In this paper the effects of salts on the formation of such complexes are described and compared with their effects on the separation of floccules.

MATERIAL AND METHODS

The proteins used for studying the changes in precipitability produced by the formation of complexes were the 'soluble euglobulin' and albumin fractions from rabbit serum. The proteins used as antigens were tomato bushy-stunt virus, and the soluble whole globulin and albumin fractions from human serum. These were all prepared by the methods previously described [Kleczkowski, 1941 *a*, *b*; Bawden & Kleczkowski, 1941].

The antisera used were against tomato bushy-stunt virus, against the whole globulin of human serum and against human serum albumin. They

were produced from rabbits by intravenous injections.

Dialysis was carried out in cellophane sacs for 3 days against distilled water changed three times a day. To prevent any bacterial decomposition of serum proteins, a little thymol was put into the sacs.

The protein solutions were heated in thin-walled tubes immersed in a water-bath.

The precipitability of heated protein solutions was measured by finding the concentration of $MgSO_4$ necessary to cause precipitation. When a precipitate was formed, it was allowed to settle by leaving the fluid undisturbed for 3 days. Then the supernatant was decanted, and the precipitate washed three times by resuspending in $MgSO_4$ solutions of an appropriate concentration and centrifuging. The N content was determined by a micro-Kjeldahl method, and the results expressed in terms of protein by multiplying by 6·25.

RESULTS

The effect of NaCl on the formation of complexes between soluble euglobulin and albumin fractions of rabbit serum

Table 1 shows the results of heating the soluble euglobulin and albumin fractions from rabbit serum separately and together, both in the presence and absence of NaCl. All the solutions were heated for 10 min. at 80° at *p*H 7·0. It will be seen that when heated in the presence of salt a product was formed in the protein mixture, whose precipitability with $MgSO_4$ differed widely from either protein fraction heated separately. The amount of the product was larger than the total euglobulin in the mixture, so that albumin as well as globulin must have contributed to its formation.

Table 1. *The effect of NaCl on interaction between globulin and albumin fractions of rabbit serum during heating*

The amount of proteins in 4 ml. of heated solutions (mg.)		The amount of precipitate formed during heating (mg.)	The amount of precipitate formed with the following concentrations of $MgSO_4$ (% saturation) (mg.)				
Euglobulin	Albumin		30	50	70	80	100
		Heating with 1% NaCl					
0	15	0	0	0	0	0	0
5	0	4	.	.	.	.	.
5	15	0	0	0	0	8	.
		Heating in salt-free solutions					
0	15	0	0	0	0	0	0
5	0	0	3·2	.	.	.	.
5	15	0	0	4	.	.	.
5	0 mixed after	0	3·2	.	.	.	.
0	15 heating						

*p*H of the dialysed solutions was adjusted to 7·0 by adding 0·1 *N* NaOH. The solutions were heated for 10 min. at 80°.

Serological precipitin tests were made by adding 1 ml. of antigen solutions to 1 ml. of antiserum solutions, so that the range of antigen/antibody ratios, indicated in the tables, was covered. The tubes were immediately placed in a water-bath at 50° with the fluid columns half immersed, so that convection currents ensured complete mixing. The appearance of floccules was taken as a positive result. The ability of treated antigens to combine with antibody without causing precipitation was measured by their ability to inhibit precipitation. After 3 hr. incubation at 50°, appropriate amounts of unchanged antigen were added to the tubes in which there were no floccules. The absence of precipitation after this addition of precipitable antigen was taken as evidence of inhibition by the treated antigen. Similarly, the ability of treated antibody to combine with antigen without causing precipitation was tested by adding untreated antiserum to the tubes in which there was no precipitation.

When the salt-free mixture of the proteins was heated, a product was also formed whose precipitability differed from that of either protein heated separately. This effect was not due to mixing the proteins, for when the two were heated separately and then mixed, their precipitabilities were the same as when tested separately. Thus during heating together both in the presence and absence of salt, there is an interaction between the two proteins leading to formation of a product with new properties. The amount of the product, however, is much greater when the heating is carried out in the presence of salt, and its precipitability then also differs more widely from that of the euglobulin heated alone. These results suggest that in the presence of salt euglobulin combines with considerable quantities of albumin, whereas in the absence of salt it combines with only small quantities to give a product whose properties differ only slightly from those of the euglobulin heated alone.

The effect of salts on the formation of serologically non-precipitating complexes between human and rabbit serum albumins during heating

The euglobulin and albumin fractions of rabbit serum differ so widely in their precipitability with $MgSO_4$ and $(NH_4)_2SO_4$, both before and after heating, that complex formation between them can be readily detected by changes in the precipitability with these salts. This method, however, can obviously not be applied to proteins with similar precipitabilities, such as the albumin fractions from two different animal sera. The formation of complexes often influences the serological reactions of protein-antigens, so that serological methods can be applied for their detection in systems where precipitation with salts is unsuitable. In the tests described below the inability of complexes to precipitate with the antisera, and also their ability to inhibit specifically the precipitation of unchanged

Table 2. *The effect of salts on the formation of non-precipitating complexes between human serum albumin and rabbit serum albumin*

Concentration of salt (normality) in heated solutions	Appearance of the solutions after heating	Precipitin test Human albumin mg. 0·5	0·25	0·12	0·06	0·03	0·015	0·0075
Unheated human albumin		−	+	+	+	+	+	+
Heating at *p*H 7·0								
R Salt-free	clear	+	+	+	+	+	+	+
$Al_2(SO_4)_3$								
R 0·00015	clear	+	+	+	+	+	−	−
R 0·0003	clear	+	+	i	i	0	0	0
R 0·0006	sl.op.	i	i	i	i	i	0	0
R 0·0012	st.op.	+	+	+	+	0	0	0
R 0·0025	pptt.	.	.	.	.	.	.	.
H 0·0006	clear	−	+	+	+	+	+	+
$MgSO_4$								
R 0·0006	clear	+	+	+	+	−	−	−
R 0·0012	clear	+	+	i	i	0	0	0
R 0·0025	sl.op.	i	i	i	i	i	i	0
R 0·005	st.op.	i	i	i	i	i	0	0
R 0·01	st.op.	+	+	+	0	0	0	0
R 0·015	pptt.	.	.	.	.	.	.	.
H 0·0025	opal.	+	+	+	+	+	+	−
Heating at *p*H 3·2								
R Salt-free	clear	+	+	+	+	+	+	+
$BaCl_2$								
R 0·05	clear	+	+	+	+	+	+	+
R 0·2	opal.	i	i	i	i	0	0	0
H 0·2	clear	+	+	+	+	+	+	+

Concentration of salt (normality) in heated solutions	Appearance of the solutions after heating	Precipitin test Human albumin mg. 0·5	0·25	0·12	0·06	0·03	0·015	0·0075
$BaCl_2$								
R 0·0006	clear	+	+	+	i	0	0	0
R 0·0012	clear	+	+	i	i	i	0	0
R 0·0025	sl.op.	i	i	i	i	i	i	0
R 0·005	st.op.	−	+	+	+	+	+	+
R 0·01	pptt.	.	.	.	.	.	.	.
H 0·0025	sl.op.	+	+	+	+	+	+	−
Na_2SO_4								
R 0·012	clear	+	+	+	i	0	0	0
R 0·05	sl.op.	+	i	i	i	0	0	0
R 0·2	sl.op.	i	i	i	i	0	0	0
H 0·2	sl.op.	+	+	+	+	+	+	+
NaCl								
R 0·006	clear	+	+	+	0	0	0	0
R 0·012	clear	+	+	i	i	0	0	0
R 0·025	sl.op.	+	i	i	i	i	0	0
R 0·05	sl.op.	i	i	i	i	i	i	0
R 0·2	sl.op.	i	i	i	i	i	i	0
R 0·3	opal.	i	i	i	i	+	+	+
R 0·6	st.op.	−	−	+	+	+	+	+
H 0·2	sl.op.	+	+	+	+	+	+	+
Na_2SO_4								
R 0·003	clear	+	+	+	+	+	+	+
R 0·006	sl.op.	+	+	i	i	0	0	0
R 0·012	opal.	i	i	i	i	i	0	0
R 0·025	pptt.	.	.	.	.	.	.	.
H 0·012	sl.op.	+	+	+	+	+	+	+

Precipitin test. 1 ml. of an antiserum to human albumin at a dilution 1/40 was added to 1 ml. of antigen solutions at various concentrations. + indicates precipitation and − no precipitation if test for inhibition was not made. Inhibition was tested by addition of 0·1 ml. of 0·03% solution of unheated human albumin to the mixtures where there was no precipitation. i indicates inhibition, 0 indicates no inhibition.

The solutions were heated for 10 min. at 83°.

R, heated solution contained 0·05% of human and 0·5% of rabbit albumin.

H, heated solution contained 0·05% of human albumin.

sl.op. slight opalescence; opal. moderate opalescence; st.op. strong opalescence; pptt. precipitation.

antigens, have been used as a test for their formation [cf. Bawden & Kleczkowski, 1941].

The formation of complexes between albumin from human serum and albumin from rabbit serum was studied, using an antiserum to human albumin. Dialysed solutions of the two albumins were adjusted to *p*H 7·0 and *p*H 3·2 by adding 0·1 *N* NaOH or HCl respectively. They were then heated separately and in mixtures containing ten times as much rabbit albumin as human albumin. They were heated for 10 min. at 83° salt-free and in the presence of various salts at different concentrations. After cooling, the solutions at *p*H 3·2 were adjusted to *p*H 7·0 by adding 0·1 *N* NaOH. All the heated solutions were diluted in physiological saline and tested serologically for their ability to give precipitation or inhibition with human albumin antiserum. The results are given in Table 2.

Human albumin heated separately at either *p*H 7·0 or 3·2, salt-free, or in the presence of all the tested salts, precipitated with the antiserum to human albumin. This specific precipitation was not affected if rabbit albumin, either unheated or heated separately, was mixed with the heated human albumin. The salt-free solutions of the mixtures of human and rabbit albumins, heated at either *p*H, also precipitated with the antiserum. Heating these mixtures in the presence of all the salts, on the other hand, produced non-precipitating complexes that inhibited the precipitation of human albumin added subsequently.

For each salt four zones of concentration can be distinguished, in each of which the effect of heating the protein mixture is different. At low concentrations no serologically demonstrable complex is formed and heated solutions are precipitated by the antiserum. In the next zone a serologically non-precipitating complex is formed. Heating in the third zone gives material that is precipitated specifically by the antiserum. This material flocculates with antiserum more quickly than the unheated human albumin and forms larger floccules. In the zone of highest salt concentration a precipitate of denatured protein separates during heating.

Hardy [1900] showed that the efficiency of salts in flocculating colloidal solutions (including proteins) is determined by the valency of the ion with the charge opposite to that of the colloidal particles. The efficiency is expressed as the reciprocal of the lowest concentration, in terms of normality, necessary to cause flocculation. Ions of higher valency are much more efficient than those of lower valency. Chick & Martin [1912] found that Hardy's law also applies to the efficiency of salts in flocculating protein solutions previously denatured by heat in the absence of salts. The results given in Table 2 show that this law also applies to the formation of complexes between two different proteins. At *p*H 7·0, i.e. on the alkaline side of the isoelectric point of heated proteins, when the protein particles are negatively charged, the efficiency of salts in causing the complex formation is determined by the valency of the cations. The lowest concentration of $Al_2(SO_4)_3$ causing the complex formation was approximately one-tenth of that of $MgSO_4$ or $BaCl_2$, and this was approximately one-tenth of that of Na_2SO_4 or NaCl. At *p*H 3·2, i.e. on the acid side of the isoelectric point of the heated proteins, when the protein particles are positively charged, the efficiency of salts in causing the complex formation depends on the valency of the anions. The lowest concentration of Na_2SO_4 causing the complex formation was approximately one-tenth of that of $BaCl_2$.

The fact that the efficiency of salts in promoting the complex formation is determined by the valency of the ion charged oppositely to the protein particles, strongly suggests that the action of salts is electrostatic. They can be pictured as acting by discharging the protein particles, thus facilitating their combination with each other, as when they cause flocculation of colloidal suspensions. The formation of protein complexes, however, is not altogether comparable with flocculation. Flocculation is caused whether salts are present during the heating or are added to the protein solutions after heating. The formation of protein complexes, however, takes place to any appreciable extent only when the mixtures are heated in the presence of salt. If salt-free mixtures of human and rabbit serum-albumin fractions are heated and salt added after cooling, no material that inhibits serological precipitation can be detected, and the heated mixture precipitates with the antiserum to human albumin in exactly the same way as human albumin heated alone.

The effect of salt on the formation of a serologically non-precipitating complex between tomato bushy-stunt virus and rabbit serum albumin during heating

Table 3 shows the influence of NaCl on the formation of serologically non-precipitating complex between tomato bushy-stunt virus and rabbit-serum albumin. In salt-free solution no demonstrable amount of the complex was formed, and the heated virus-albumin mixture behaved serologically like a solution of the virus heated alone. When heated in the presence of salt, a non-precipitating complex was formed, and the lowest concentration of NaCl causing the complex formation was approximately equal to the lowest NaCl concentration causing formation of the complex between human- and rabbit-serum albumins (cf. Table 2).

With this system also, the addition of salt to the virus-albumin mixture previously heated salt-free did not cause the formation of any demonstrable quantities of a non-precipitating complex.

Table 3. *The effect of NaCl on the formation of non-precipitating complexes between tomato bushy-stunt virus and rabbit serum albumin*

Concentration of NaCl (normality) in heated solutions	Appearance of the solutions after heating	Precipitin test Dilutions of the antiserum 1/20	1/40	1/80	1/160	1/320	1/640
Unheated bushy-stunt virus		+	+	+	+	+	–
Heated virus-albumin mixture:							
0·012	clear	+	+	+	+	–	–
0·025	sl.op.	0	0	0	i	i	–
0·05	sl.op.	0	i	i	i	i	–
0·1	opal.	i	i	i	i	i	–
Salt-free	clear	+	+	+	+	+	+
The virus heated alone:							
0·1	st.op.	+	+	+	+	+	+

Precipitin test. 1 ml. of every antigen preparation diluted 1/10 in physiological saline was mixed with 1 ml. of an antiserum to bushy-stunt virus at varying dilutions.
Inhibition was tested by adding 0·1 ml. of 0·05% solution of the unheated virus.
The solutions were heated for 10 min. at 83° at *p*H 7·0.
Virus-albumin mixture contained 0·05% of bushy-stunt virus and 0·5% of rabbit serum albumin.
The virus alone was used as 0·05% solution.
The symbols as in Table 2.

The effect of salt on the formation of a serologically non-precipitating complex between soluble human serum globulin and rabbit serum albumin during heating

Table 4 shows that when the water-soluble fraction of human-serum globulin was heated with rabbit-serum albumin, rather different results were obtained. In the presence of salt the system resembled the other two described above; a serologically non-precipitating complex was formed which inhibited the precipitation of the unheated globulin added later. The mixture heated in the absence of salt, however, also failed to precipitate with the antiserum, although it did not give the phenomenon of inhibition. When the globulin was heated alone, it still precipitated specifically with the antiserum whether rabbit albumin was later added or not. Thus the change in behaviour of the salt-free mixture was a result of an interaction between the two proteins during heating.

It has been shown above, by measuring changes in the precipitability with $MgSO_4$, that there was some combination between rabbit euglobulin and albumin when a mixture was heated salt-free, although much less than when heated in the presence of NaCl. The results in Table 4 suggest that a complex was also formed between human globulin and rabbit albumin when heated salt-free. It appears that the complex was formed in an amount sufficient to destroy serological precipitability but not sufficient to produce the inhibition phenomenon, which was well pronounced when NaCl was present during heating and the complex formation occurred to a greater extent.

Table 4. *The effect of NaCl on the formation of non-precipitating complexes between soluble human serum globulin and rabbit serum albumin*

Treatment of human globulin	Appearance of the solutions after heating	Precipitin test Human globulin mg. 0·5	0·25	0·12	0·06	0·03	0·015	0·0075
Unheated		+	+	+	+	+	+	+
Heated with albumin in 1% NaCl	st.op.	i	i	i	i	0	0	0
Heated with albumin salt-free	clear	0	0	0	0	0	0	0
Heated alone in 1% NaCl	pptt.	.	.	.	.	.	.	.
Heated alone salt-free	st.op.	+	+	+	+	–	–	–

Precipitin test. 1 ml. of an antiserum to human globulin at a dilution 1/10 was added to 1 ml. of antigen solutions at various concentrations.
Inhibition was tested by adding 0·1 ml. of 0·06% solution of unheated soluble human globulin.
The solutions were heated for 10 min. at 83° at *p*H 7·0.
In all heated solutions concentration of human globulin was 0·05% and that of rabbit albumin 0·5%.
The symbols as in Table 2.

The effect of salt on the formation of non-precipitating complexes between antibodies and unspecific serum proteins during heating

Antibodies can undergo initial stages of heat denaturation without losing their ability to combine with their antigens, and, like protein-antigens, when heated in the presence of other proteins undergoing denaturation simultaneously, they can combine with them to form complexes [Kleczkowski, 1941*b*]. If combined with sufficient albumin, they do not precipitate their antigens, but, as they still combine with them, they interfere with the action of precipitating antibodies. The degree of this interference depends on the nature of antigen, being large with 'O'-type antigens and small with 'H'-type antigens. Heating whole antisera can lead to the formation of such complexes produced by heat-changed antibody particles combining with serologically unspecific proteins present in the sera.

To test the effect of salt on the formation of such antibody complexes, dialysed antisera to human-serum albumin and to tomato bushy-stunt virus were used. The precipitate of insoluble globulin formed during dialysis was filtered off and the filtrate was used as test solution. This involved loss of antibodies, but sufficient remained in solution for the test. The results of heating the dialysed antisera with and without addition of NaCl are given in Table 5.

DISCUSSION

All the antigens used in this work belong to the group of proteins which, when heated in the presence of salt with serologically unspecific serum albumin, combine with it to form serologically non-precipitating complexes. In spite of their uniform behaviour in the presence of salt, when heated salt-free they behave differently. Bushy-stunt virus, and human-serum albumin, heated with rabbit-serum albumin in salt-free solutions did not form any demonstrable amounts of non-precipitating complexes; they still precipitated with their antisera exactly as if they had been heated alone. On the other hand, the water-soluble fraction of human-serum globulin heated salt-free in the presence of rabbit-serum albumin formed a non-precipitating complex, although to a smaller extent than in the presence of salt. This complex did not precipitate with the antiserum, but it differed from that formed in the presence of salt as it did not show the presence of inhibition. It is difficult to account for these differences satisfactorily, although various tentative explanations can be offered. Susceptibility to the presence of traces of salts, which might have remained after dialysis, may vary with different proteins, so that complex formation could proceed in globulin-albumin mixtures but not in the two other protein systems. The differences may be apparent rather than real, for only the precipitin test was used to detect the formation of the complexes. Therefore, if the different antigens vary in their sensitivity to the presence of the complexes, they may have been formed equally in all three systems in the absence of salts, but only with globulin may it have been sufficient to prevent precipitation. It is, however, equally possible that the globulin differs from albumin and the virus when heated in the absence of salt, and that the first can combine with rabbit-serum

Table 5. *The effect of NaCl on the formation of non-precipitating complexes between antibodies and unspecific serum proteins*

	The antiserum to human albumin Human albumin (mg.)						The antiserum to bushy-stunt virus Dilutions of the antiserum					
Treatment of the antisera	0·25	0·12	0·06	0·03	0·015	0·0075	1/10	1/20	1/40	1/80	1/160	1/320
Unheated	+	+	+	+	+	+	+	+	+	+	–	–
Heated in 1% NaCl	0	0	i	i	i	i	i	i	i	i	0	0
Heated salt-free	+	+	+	+	+	+	i	i	i	0	0	0

Dialysed antisera were heated for 10 min. at 80° at *p*H 7·0 at a dilution 1/10 in distilled water with and without addition of 1% NaCl.

Precipitin test. The antiserum to human albumin was tested by mixing 1 ml. of the antiserum diluted 1/10 with 1 ml. of solutions of human albumin at various concentrations. The inhibition was tested by adding 0·1 ml. of undiluted dialysed antiserum.

The antiserum to bushy-stunt virus was tested by mixing 1 ml. of 0·005% solution of the virus with 1 ml. of various dilutions of the antiserum. The inhibition was tested by adding 0·1 ml. of the dialysed antiserum diluted 1/2.

The symbols as in Table 2.

Both antisera behaved similarly when heated in the presence of salt, forming non-precipitating and inhibiting complexes. They showed, however, differences after heating salt-free. The antiserum to human albumin still precipitated its antigen, whereas the antiserum to bushy-stunt virus did not precipitate and instead gave inhibition, although the 'inhibition titre' was slightly lower than that given by the same antiserum after heating in the presence of salt.

albumin in these conditions whereas the others cannot.

Whether or not the presence of salt is necessary to start the combination of two proteins, it certainly greatly increases it. As in causing flocculation, the efficiency of different salts in influencing complex formation depends on the valency of the ion with the charge opposite to that of the protein particles. The two processes, however, are not identical. If the protein is denatured by heating in the absence of salts, the subsequent addition of salt causes flocculation. By contrast, salts influence complex formation only if they are present in protein mixtures during heating, for the addition of salt to a protein mixture heated salt-free does not lead to formation of any measurable amount of serologically non-precipitating complexes. This difference could be explained by assuming that individual proteins differ in their rates of aggregation, and that these rates are much greater than rates of denaturation. Then, if salt is present during heating, particles of different proteins would be able to combine with one another as soon as they are denatured, but if salt is added to a mixture of proteins already denatured by heating in salt-free solution, each protein would tend to aggregate separately.

The results with antibodies almost exactly parallel those with antigens. The two antisera used were to bushy-stunt virus and to human-serum albumin. When heated in the presence of salt, these behave similarly, the antibodies forming non-precipitating complexes which inhibit precipitation. In the absence of salts the two antisera behaved differently. The virus antiserum again ceased to precipitate and instead inhibited precipitation, though less strongly than when heated with salt, whereas the antiserum to human albumin still precipitated. This difference at first sight suggests differences in the heat stabilities of the two antisera. A similar difference between the behaviour of antisera to 'O' and 'H'-type antigens is well known and was for long interpreted as evidence that these antigens produced antibodies with different properties. It has now been shown, however, that antibodies to antigens of both types undergo similar changes when heated, and that the difference in their behaviour occurs because the precipitability of the antigens is affected differently by the presence of antibodies changed into non-precipitating complexes [Kleczkowski, 1941*b*; Bawden & Kleczkowski, 1941, 1942]. Similarly, the difference between the behaviour of dialysed antisera to human albumin and to bushy-stunt virus respectively, may be because the two antigens are affected differently by the presence of non-precipitating antibody complexes, which are probably formed to a small extent when both antisera are heated in the absence of salts.

SUMMARY

The formation of complexes between different proteins, undergoing heat denaturation together, is greatly influenced by the presence of salts. In the absence of salts only mixtures containing water-soluble serum globulin formed any detectable amount of complexes.

The efficiency of different salts in promoting the complex formation, expressed as the reciprocal of the lowest effective concentration, follows Hardy's law. Ions of higher valency are much more effective than those of lower valency. On the alkaline side of the isoelectric point of the proteins, the efficiency of salts is determined by the valency of the cations, and on the acid side by the valency of anions.

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The Influence of Halide Concentration on the Metabolism of *Penicillium sclerotiorum* van Beyma

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In a previous communication [Curtin & Reilly, 1940], the isolation and properties of sclerotiorine, a chlorine-containing metabolic product of *Penicillium sclerotiorum* van Beyma, were described. Reference was also made to a second product, a red pigment retained by the mother-liquors of crystallization. Work on the constitution of sclerotiorine was somewhat hampered by the small yield, 2% of