the existence of a third isomer must be accounted for on other grounds. It is possible of course that the third form may have the oxygen ring linkage on some other than the gamma carbon atom. This is the suggestion which Fischer[1] has recently made in connection with his discovery of a third modification of methyl glucoside which he obtained in the form of a distillable sirup. As mentioned, Dr. Johnson and myself are continuing the investigation of the isomerism of the three galactose pentacetates.

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[CONTRIBUTION FROM THE NORTHWESTERN UNIVERSITY MEDICAL SCHOOL.]

ON THE COMBINATION OF PROTEIN WITH HALOGEN ACIDS.[2]

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Received April 28, 1915.

Certain classes of combinations between protein bodies and halogen acids, and, in particular, hydrochloric acid, have been studied and frequently described. Before the full recognition of the fact that the proteins may act as basic bodies and hold these acids in salt forms, the distinction between such salts and substitution compounds was not clearly made. Hydriodic acid, for example, forms salts with egg albumin, but it forms also substitution products in which the iodine replaces hydrogen in nucleus groups. In this manner relatively large amounts of iodine may be added, but the products lose the properties of proteins.

In the production of these compounds and in the determination of the proportions in which proteins and halogen acids unite, a number of quite different processes have been employed. The results vary with the process and we are still without a satisfactory answer to some phases of the general question. Among the earliest papers describing definite combinations between proteins and acid those of Paal[3] may be referred to. Paal produced compounds which he considered as definite salts of peptones, containing from 10 to 15% of HCl. These were made by treatment of egg albumin with diluted acid and also by digestion of the protein by acid and pepsin. In these cases the salts were separated in comparatively pure form, but in most inquiries on the subject the aim has been to determine the ratio of combination between protein and halogen, rather than the isolation of the actual compounds. This has been done, usually, by finding the amount of acid held by a given weight of protein, when an excess is added and this excess measured by the aid of an appropriate indicator. In this manner, for example, Osborne[4] showed, by use

[1] *Ber.*, 47, 1980 (1914).

[2] Presented at the New Orleans meeting of the American Chemical Society, April 2, 1915.

[3] C. Paal, *Ber.*, **25**, 1202 (1892); **27**, 1827 (1894).

[4] THIS JOURNAL, 21, 477 and 486 (1899).

of tropaeolin, that 1 g. of certain proteins will bind from 9 to 13 cc. of 0.1 *N* HCl. With the pure protein edestin, the mean amount was 12.7 cc. or 46.3 mg. This is 4.63% of the protein weight. Panormow[1] described definite molecular combinations of albumins with several acids which were obtained by mixing the proteins with dilute acids and precipitating by alcohol. The weights of hydrochloric and other acids combined in this manner appear from the analyses, however, to be rather low. In one case a hydrochloride contained HCl amounting to about 3.2% of the weight of the protein.

By aid of titrations with Guenzburg's reagent, Hoffa found that serum albumin will combine with about 6.3% of its weight of HCl, while the albumoses from egg albumin will combine with about 7.7%. These values become greatly increased as the proteins undergo digestion.[2]

Spiro and Pemsel[3] followed a different method to determine the amount of acid which may be combined by proteins. This was based on the fact that the hydrochlorides or other combinations may be salted out as are the proteins themselves. By adding an excess of 0.2 *N* or 0.1 *N* acid to the protein solution, then a sufficient amount of ammonium sulfate solution for precipitation, the uncombined excess of acid may be titrated in the filtrate. Results are given for egg and serum albumins. For the former the amount of HCl combined was about 5% of the protein weight, and for the latter about 7% in the mean.

By following the general method of Cohnheim and Krieger, results were found by Erb[4] which show a far greater maximum combining power of HCl than is indicated by the work of other observers. These results cannot be reconciled with the values obtained by Spiro and Pemsel, who did not observe any marked increase in the amount of HCl held when the weight mixed with the protein is increased. In the Erb experiments the amount of HCl held by 1 g. of serum albumin increases from 104 mg. to 204 mg. when the volume of 0.1 *N* acid used in solution is quadrupled. However, by use of a method identical in principle v. Rhorer[5] reached the conclusion that the acid held by the protein does not vary as claimed by Erb. He showed, further, that the method followed by Erb could not be expected to yield accurate results. This conclusion seems justified by the facts.

We have sought to throw some light on the problem by working in a

[1] *J. russ. phys.-chem. Gesell.*, 31, 556 (1899) and 32, 249 (1900); through *Jahresb. Fortschr. Thier-Chemie*, 29, 8 (1899) and 30, 6 (1900).

[2] *Jahresb. Fortschr. Thier-Chemie*, 30, 52 (1900).

[3] *Z. physiol. Chem.*, 26, 233 (1898).

[4] *Chem. Centr.*, 1901, II, 359. Cohnheim, "Chemie der Eiweisskoerper," 126.

[5] *Arch. ges. Physiol.*, 90, 368 (1902).. See also the discussion by Robertson, in *Ergebnisse der Physiologie*, 10, 216 (1910), where there is a very full citation of the literature.

very simple manner and by methods which at first sight might not be expected to give definite results. Our investigations grew out of some experiments intended to show the extent of combination between the acids and solid or coagulated proteins, and the behavior of these compounds on hydrolysis.[1] In our experiments we have added known volumes of diluted acids to known weights of proteins and have dried the mixtures at a low temperature or over sulfuric acid, followed by drying over solid alkali. It will be seen that reasonably constant results may be secured in this way which have some meaning.

While the titration of the excess of free acid standing over a mixture of acid and protein cannot give a correct measure of the acid which may be held, because of the ready dissociation of such compounds with liberation of acid, and because, also, of the behavior of available indicators in presence of unaltered protein, still the results in this way have some value for comparison. Some of the results reported by other workers, and referred to above, were thus secured, for example, those by Osborne cited.

We made mixtures of coagulated egg albumin with 25 cc. of 0.1 *N* HCl and 25 cc. of water and treated them as described below. The albumin used was in the form of dried, soluble, egg albumin which was weighed out in multiples of gram portions, dissolved and poured into a large excess of boiling distilled water containing a little dilute acetic acid. By pouring slowly and stirring constantly it is possible in this way to secure a very fine uniform coagulum. For each gram of weighed egg powder the protein in the coagulum amounts to 700 mg. Methyl orange was finally added to the mixed protein and acid, and the free acid determined. The difference between this and the acid added is calculated as acid held. The results are of course below the truth, somewhat. Some of these residual titrations were made at once and some after certain intervals. In some cases the acid solution and protein were mixed by stirring, without further agitation. In other experiments the mixtures were actively shaken in flasks and then allowed to stand, while, finally, in other experiments, the protein and acid were well rubbed together in a mortar. Table I gives the results secured.

The table discloses the fact that, even after long standing, the combination between acid and protein is incomplete if the mixture is simply stirred. Even after thorough shaking the same is true, but constant results come speedily when the mixture are well rubbed in the mortar. We secure practically the same end result from the solid protein, in this manner, that others have reported for protein solutions. The egg albumin combines with about 3% of its weight of HCl, when heat is not applied, and this represents a somewhat constant value for the combination as

[1] See paper in a recent number of THIS JOURNAL, by one of us, "On the Physiological Behavior of Combined Hydrochloric Acid," **37**, 1333.

measured by indicators. For other proteins the results are not necessarily the same, of course.

TABLE I.

	Coag. from gm. egg.	Wt. of HCl held. At once.	After 30 min.	After 60 min.	After 90 min.
Mixture made without shaking.....	1	0.0065	0.0112	0.0183	0.0189
	2	0.0102	0.0205	0.0284	0.0339
	3	0.0204	0.0412	0.0509	0.0525
Mixture made by active shaking.....	1	0.0076	0.0175	0.0200	0.0211
	2	0.0193	0.0295	0.0394	0.0423
	3	0.0306	0.0554	0.0605	0.0660
Mixture made by rubbing in mortar....	1	0.0206	0.0222	0.0215	0.0219
	2	0.0425	0.0429	0.0419	0.0430
	3	0.0690	0.0700	0.0773	0.0740

By heating the proteins with dilute acid solutions on the water bath and evaporating slowly to dryness an increase in weight follows which corresponds to the addition of the acid and not to addition of acid plus water, which would be the case if hydrolysis had taken place. This is illustrated by the following experiments in which a constant weight of fibrin was mixed with increasing volumes of 0.2 *N* HCl, evaporated slowly to dryness, and weighed after heating to practically constant weight in the electric oven to 105°. The dry weight of the fibrin alone was 0.677 g. After weighing, the residue was mixed with carbonate and nitrate and fused. In the melt the HCl was determined by the Volhard method.

TABLE II.

No.	Cc. 0.2 *N* HCl.	Mg. HCl.	Dry wt.	Inc. wt	HCl found.
1.....	20.0	146.0	0.740	0.063	0.0635
2.....	15.0	109.5	0.743	0.066	0.0642
3.....	12.5	91.2	0.745	0.068	0.0640
4.....	10.0	73.0	0.742	0.065	0.0666
5.....	7.5	54.8	0.730	0.053	0.0519
6.....	5.0	36.5	0.704	0.027	0.0330
7.....	2.5	18.3	0.696	0.019	0.0176

The determination of the chlorine is much more accurate than is that of the dry weight. It is somewhat difficult to bring the protein, with or without the acid, to a constant weight, but the results show a satisfactory degree of constancy. In Nos. 1, 2, 3 and 4 it is evident we have added a great excess of acid, and that the excess was expelled without adding water by hydrolysis. An amount of acid below that in No. 5 is insufficient to saturate the protein, under these conditions. The mean increase in weight for the first four mixtures is 0.0655, while the mean HCl addition in the same 0.0646. It is evident that we have no water addition here. In similar experiments made with egg albumin the mean increase in weight was 0.0680 and the mean HCl content 0.0704.

With this point established the greater number of experiments were concerned with the extent of the halogen acid addition, rather than with the gross weight increase. The experiments were made essentially in the same manner, using casein, fibrin and egg albumin.

Casein and HCl.

TABLE III.

In each test 750 mg. anhydrous casein employed. Evaporated over live steam.

No.	Cc. 0.2 *N* HCl.	Mg. HCl.	Mg. HCl held.	Mean of first four.
1.....	20.0	146.0	69.5	70.9
2.....	15.0	109.5	72.4	
3.....	12.5	91.2	70.3	
4.....	10.0	73.0	71.4	
5.....	7.5	54.8	52.0	
6.....	5.0	36.5	34.9	
7.....	2.5	18.3	17.2	

TABLE IV.

750 mg. of anhydrous casein plus HCl.

No.	Cc. 0.2 *N* HCl.	Mg. HCl.	Mg. HCl held.	Mean of first four.
1.....	20.0	146.0	66.6	67.8
2.....	15.0	109.5	68.8	
3.....	12.5	91.2	67.5	
4.....	10.0	73.0	68.5	
5.....	7.5	54.8	45.8	
6.....	5.0	36.5	32.9	
7.....	2.5	18.3	16.4	

As several experiments suggested that a high temperature might have the effect of causing the addition of too much acid, accompanied by some hydrolysis, the later tests were made by evaporating on a water bath, and slowly. Table IV shows the results secured by this condition of evaporation.

With the lower temperature the acid held is somewhat less and amounts to 9.04% of the weight of the casein. It is clear that no excess of acid is added in the cases where an excess of acid was mixed with the casein at the outset. The capacity for combination seems to be a constant in this respect, but apparently varies with the temperature. This result, which is confirmed by many others, seems to disprove completely the view of Erb, referred to above, that the amount of acid taken up by the protein increases with the amount added.

Fibrin and HCl.

The fibrin used for these tests was thoroughly washed until white, mixed with toluene and pressed out as dry as possible, after passing a number of times through the meat chopper. The mass so prepared holds enough toluene to keep some weeks unchanged, at a low temperature. It is not necessary to freeze it. The experiments were made as with the casein, by evaporating slowly on a water bath. Two independent sets of experiments, A and B, are given in Table V.

The results here for the larger acid concentrations are very regular and close to a mean value. They are somewhat higher than the corresponding results for the casein and show, as we found there, that no increase in the amount of HCl combined follows from increase in amount added to the fibrin.

TABLE V.

2.5 g. fibrin = 750 mg. protein, plus HCl.

No.	Cc. 0.2 *N* HCl.	Mg. HCl.	A. Mg. HCl held.	B. Mg. HCl held.	Mean of first four.
1	20.0	146.0	71.1	70.1	70.8
2	15.0	109.5	70.0	70.3	
3	12.5	91.2	71.1	72.1	
4	10.0	73.0	71.1	70.3	
5	7.5	54.8	50.7	51.5	
6	5.0	36.5	31.9	32.8	
7	2.5	18.3	16.4	17.3	

Egg Albumin and HCl.

We have made at different times a large number of tests as to the rate of combination between the acid and this common protein. The egg used for the purpose was referred to above, when another experiment was described. It was part of a large lot of clear dried egg white of Chinese origin which was selected because of its solubility and low color, and which we have used as a standard preparation for a number of years. In these experiments a known amount was weighed and coagulated, as explained above, and small portions of the coagulum, to correspond to 750 mg. of anhydrous protein, taken for each test. Volumes of 0.2 *N* HCl were added as in the other tests and the mixtures evaporated slowly to dryness on the water bath. After this the residue was dried at 105° to a constant weight. As the coagulum holds a large amount of water the dry weights taken are not as accurately defined as with the casein or the fibrin.

Two sets of experiments are here given, A and B, Table VI.

TABLE VI.

1.07 g. egg = 750 mg. protein, plus HCl.

No.	Cc. 0.2 *N* HCl.	Mg. HCl.	A. Mg. HCl held.	B. Mg. HCl held.	Mean of first four.
1	20.0	146.0	80.3	70.3	77.7
2	15.0	109.5	84.0	80.3	
3	12.5	91.2	81.8	83.6	
4	10.0	73.0	70.3	71.2	
5	7.5	54.8	51.5	52.9	
6	5.0	36.5	32.8	35.0	
7	2.5	18.3	17.3	16.0	

The values for the egg combination are not as uniform as were the others, but it is clear that no excess of HCl is held by the higher concentrations. Egg albumin seems to have a higher binding power than has fibrin or casein, the relations, calculated for the gram basis, being:

Casein, 90.4, or 9.04%; fibrin, 94.4, or 9.44%; egg albumin, 103.6, or 10.36%.

These values are much higher than are those found by means of indicators, and undoubtedly have as definite a meaning. Before going into any further discussion concerning them, the results given in corresponding experiments with hydrobromic acid and hydriodic acid will be given.

Hydrobromic Acid and Protein.

Portions of the three proteins were treated with 0.2 *N* HBr exactly in the manner given for HCl. The bromine was found after fusion of the dry residue.

TABLE VII.

750 mg. of anhydrous casein plus HBr.

No.	Cc. 0.2 *N* HBr.	Mg. HBr.	Mg. HBr held.	Mean of first four.
1	30.0	485.7	273.0	273.9
2	27.5	445.2	274.0	
3	25.0	404.8	275.0	
4	20.0	323.8	273.5	
5	15.0	242.8	227.4	
6	12.5	202.4	193.8	
7	10.0	161.9	153.4	
8	7.5	121.4	116.8	
9	5.0	80.9	79.1	
10	2.5	40.5	39.7	

TABLE VIII.

2.5 g. fibrin = 750 mg. protein plus HBr.

No.	Cc. 0.2 *N* HBr.	Mg. HBr.	Mg. HBr held.	Mean of first four.
1	30.0	485.7	295.0	291.8
2	27.5	445.2	286.0	
3	25.0	404.8	297.0	
4	20.0	323.8	289.3	
5	15.0	242.8	226.8	
6	12.5	202.4	190.3	
7	10.0	161.9	150.9	
8	7.5	121.4	114.7	
9	5.0	80.9	78.7	
10	2.5	40.5	40.4	

TABLE IX.

1.07 g. egg albumin powder = 750 mg. protein plus HBr.

No.	Cc. 0.2 *N* HBr.	Mg. HBr.	Mg. HBr held.	Mean of first three.
1	30.0	485.7	376.5	375.3
2	27.5	445.2	378.7	
3	25.0	404.8	370.7	
4	20.0	323.8	309.2	
5	15.0	242.8	224.7	
6	12.5	202.4	179.7	
7	10.0	161.9	147.8	
8	7.5	121.4	109.2	
9	5.0	80.9	76.5	
10	2.5	40.5	39.6	

The above results show that hydrobromic acid is taken up in large quantity by the three proteins and in amounts relatively much greater than is hydrochloric acid. For 1 g. of the proteins the acid combination is as follows:

Casein, 365 mg. or 36.5%; fibrin, 398 mg. or 38.9%; egg albumin, 500 mg. or 50%.

It is evident that the combination cannot be on the same basis as that of the hydrochloric acid, as the amounts combined are not in the proportion of 36.5 to 80.9. But the weights combined with 1 g. of protein stand in the same order for the two acids, as seen by these figures, the amount held by the casein being called 100.

HCl:	Casein, 100	Fibrin, 104.4	Egg albumin, 114.6
HBr:	100	106.6	137.0

For the casein the HCl and HBr combined are in the proportion of 90.4 mg. to 365 mg. for the gram of protein, or nearly in the ratio of 5 to 9 molecules. For the HBr combination with fibrin the ratio is a little higher with reference to the HCl. For the egg albumin the HCl and HBr combinations stand to each approximately in the proportion of 5 to 11 molecules. These numbers probably have some significance, which will be taken up below.

Hydriodic Acid and Protein.

By the same general plan combinations between the three protein bodies and hydriodic acid were made. But a little study showed that the constancy in combining ratios found with the other acids is lacking here. When this acid is added to prepared proteins and the mixture evaporated the increase in weight is roughly in agreement with the iodine taken up, calculated as HI. But sometimes there is a discrepancy, and the total increase in weight may even be less than the added iodine. This can be the case only when something else is driven out of the protein molecule, that is, when a substitution, as well as an addition follows. This is seen from Tables X and XI. In the experiments made with egg and fibrin these results were secured.

TABLE X.

1.07 g. egg albumin powder equivalent to 750 mg. protein and 806 mg. dry residue plus HI.

No.	Cc. 0.2 *N* HI.	Mg. HI.	Dry residue.	Inc. weight.	Mg. HI held.
1	30.0	768	1400	594	588
2	25.0	640	1278	472	463
3	20.0	512	1185	379	381
4	15.0	384	1111	305	287
5	12.5	320	1082	276	245
6	10.0	256	1014	208	197
7	7.5	192	985	179	161
8	5.0	128	919	113	108
9	2.5	64	866	60	53

In four additional experiments with the largest amount of 0.5 *N* acid added, 30 cc., the weight increase and HI fixed were as follows:

Increase in weight	603	588	581	572	Mean,	586
HI held	589	561	568	583	Mean,	575

It is somewhat difficult to dry these mixtures to constant weight, and this may account for part of the irregularity. In the course of the evaporation and final drying they become dark, which suggests the liberation of iodine, but treatment with carbon disulfide or chloroform fails to bring anything into solution. The following results were secured by repeated experiments with smaller weights of iodine added to the constant weight of egg:

TABLE XI.

1.07 g. egg powder = 750 mg. protein plus HI.

Cc. 0.2 *N* HI.	Mg. HI.	Inc. wt.	Mg. HI held.*
25	640	436	410
25	640	442	478
		439	444
20	512	415	379
20	512	380	400
20	512	390	394
		395	391
15	384	310	295
15	384	299	298
		305	397

In the first of the above tests in which the 30 cc. of 0.2 *N* HI was added, the amount held by the protein is over 76% of the weight of the protein. This cannot be accurately titrated by normal alkali, as is the case with the hydrochloric acid combinations of the egg albumin and other proteins. In these combinations the titration results and the chlorine determinations with silver nitrate, after fusion, agree. It appears, therefore, that the HI combinations by heat are of an order different from those of the HCl compounds. But the combinations made without heat are apparently similar, as will be shown below.

TABLE XII.

2.5 g. fibrin = 750 mg. dry protein plus HI.

No.	Cc. 0.2 *N* HI.	Mg. HI.	Mg. HI held.
1	30.0	768	595
2	25.0	640	409
3	20.0	512	392
4	15.0	384	303
5	12.5	320	253
6	10.0	256	210
7	7.5	192	153
8	5.0	128	102
9	2.5	64	59

It has been shown in a previous paper by one of us[1] that HI combines with casein under the same conditions and to yield compounds containing for 1 g. of anhydrous casein about 575 mg. of iodine, calculated as HI. This appeared to represent the maximum combining power. Like the other combinations, these possessed an ocher color, suggesting a product resembling the iodalbose of Weyl.[2] This product was prepared by add-

[1] THIS JOURNAL, **29**, 1334 (1907).

[2] *Z. physiol. Chem.*, **68**, 236 (1910).

ing egg albumin to concentrated hydriodic acid at water bath heat, and pouring the mixture into an excess of water. The ocher precipitate which formed was purified by solution in alkali and reprecipitation. The iodine is firmly fixed in the product, which seemed to be lacking in some of the characteristic protein reactions. In all our experiments the iodine was used only in the form of dilute HI solution and in absence of heat no ocher-red color was developed. The concentration of the dilute acid in evaporation possibly leads to the same or similar compounds.

Some later experiments on the combination between casein and hydriodic acid, carried out as were those for the other proteins, gave the following results. The amount of iodine held is much larger than in the earlier experiments, but whether by addition or substitution is not clear. The iodine is calculated as HI.

TABLE XIII.

750 mg. dry casein plus HI.

No.	Cc. 0.02 *N* HI.	Mg. HI.	Inc. in weight.	Mg. HI held.
1	30.0	768	601	587
2	25.0	640	400	394
3	20.0	512	393	379
4	15.0	384	329	306
5	12.5	320	299	242
6	10.0	256	229	202
7	7.5	192	141	137
8	5.0	128	112	100

Most of the compounds of iodine and protein described in the literature are substitution products, and are obtained by the action of the halogen, as solid, tincture or in KI solution. The products contain from about 5 to 15% iodine, but show reactions deviating from those of proteins in some respects. Such compounds have been described by Hofmeister,[1] Blum,[2] Liebrecht,[3] Hopkins and Pinkus,[4] Kurajeff,[5] Pauly and Gundermann[6] and others and are not to be confounded with the salts secured by treatment with weak HI. Because of the easy decomposition of the latter, however, and its marked reducing properties, iodine is liberated to be carried into the molecule.

With the hope of throwing further light on the nature of the reaction, we have repeated a number of the above experiments in such a manner as to remove the excess of acids at a low temperature. This was accomplished by adding the 0.2 *N* acid to the solid protein as before, and allowing the mixtures to stand some weeks over sulfuric acid to remove

[1] *Z. physiol. Chem.*, 24, 159 (1898).
[2] *Ibid.*, 28, 288 (1899).
[3] *Ber.*, 30, 1824 (1897).
[4] *Ibid.*, 31, 1312 (1898).
[5] *Z. physiol. Chem.*, 26, 462 (1898).
[6] *Ber.*, 41, 3999 (1908) and 43, 2243 (1910).

most of the water. The small evaporating dishes holding the residues were then placed in bell jars over powdered sodium hydroxide to absorb acid vapors. In this manner practically all of the acid was removed, and most of the water. The mixtures with HCl and HBr evaporated down without much color, but the HI mixtures became reddish brown, as before. A short, final drying in the air oven was necessary to bring to constant weight. Table XIV shows the final weights and the halogen content of the products.

Only the larger volumes of acid were added in these experiments.

TABLE XIV.— COMBINATION OF PROTEIN WITH HCl, HBr AND HI, AND EVAPORATION AT LOW TEMPERATURE. THE EQUIVALENT OF 750 MG. OF ANHYDROUS PROTEIN USED IN EACH CASE.

No.	Cc. 0.2 *N* HCl.	Mg. HCl.	Inc. in wt.	Mg. HCl held.	Cc. 0.2 *N* HBr.	Mg. HBr.	Inc. in wt.	Mg. HBr held.	Cc. 0.2 *N* HI.	Mg. HI.	Inc. in wt.	Mg. HI held.
1. Casein.....	30	219.0	73	71	30	485.7	265	270	30	768	429	426
2. Casein.....	25	182.5	81	72	25	404.8	274	268	25	640	400	415
3. Casein.....	20	146.0	74	70	20	323.8	294	271	20	512	468	420
4. Casein.....	15	109.5	73	73					15	384	382	371
			Mean,	71.5			Mean,	269.6			Mean,	420
5. Fibrin.....	25	182.5	71	63	25	404.8	289	280	30	768	450	430
6. Fibrin.....	20	146.0	65	62	20	323.8	272	280	25	640	402	311
7. Fibrin.....	15	109.5	69	63	15	242.8	272	274	20	512	405	404
			Mean,	62.6			Mean,	278			Mean,	415
8. Egg alb.....	25	182.5	77	71	25	404.8	310	348	30	768	437	433
9. Egg alb.....	20	146.0	77	73	20	323.8	292	310	25	640	487	416
10. Egg alb.....	15	109.5	65	70	15	242.8	224	232	20	512	413	390
			Mean,	71.3			Mean,	329			Mean,	413

These results are extremely interesting, as they show the extent of combination without the aid of heat. The final temperature of drying to constant weight in the electric oven was not over 75°, and this heat was not applied until all acid vapors had been absorbed in the desiccators. It will be seen that the results are very close to those secured by evaporating the acid and protein mixtures in the water bath and drying at 105°.

It is evident, therefore, that the proteins and acids unite in this manner in proportions which are apparently definite, but not in proportion to the molecular weights of the acids. The power of combination is possibly connected with the volatility of the acid, since we find that HI unites with the proteins in proportion greater than does HBr, and this, in turn, greater than HCl. Nothing is shown in the HI column of the last table which would suggest that the halogen acid addition is accompanied by the loss of water. That is, no hydrolysis appears to have taken place,

but the reaction follows as would that between glutaminic acid and hydrochloric acid, for example. In the complex protein molecules there are many of these amino groups available and the number of acid molecules which may be united to them apparently depends on the "strength" of the acid, as measured by its lower volatility.

Table XIV discloses the important fact that we have a constant combination for the hydriodic acid, when amounts above 15 cc. of the 0.2 *N* solution are used with 750 mg. of protein. For 1 g. of each protein about 553 mg. of HI goes into combination, and this evidently represents a maximum under the conditions. When heat is applied much more may be held in some form.

These halogen acid combinations, whether made by simple action of the acid on the solid protein or by this treatment followed by evaporation, undergo a rather marked degree of dissociation by contact with water. The dry hydrochloride of egg albumin gives up its acid and on this account the compound has been used to some extent as an aid to digestion. The rate of dissociation is, however, slow. Some information on this point is given in a recent publication from this laboratory in which the hydrogen concentration of HCl separated from egg albumin was measured by the gas chain method.[1] In the treatment of a given weight of a protein hydrochloride with successive equal volumes of water the acid strength of the dissociated portion gradually diminishes.

It has been shown above that the behavior of hydriodic acid with protein when evaporated is different from the behavior of the corresponding chlorine and bromine acids. But the combination as measured by indicator titration is of the same order, as shown by these results. Portions of the three substances corresponding to 750 mg. of dry protein were rubbed up in a mortar with 10 cc. of 0.2 *N* acid and washed into flasks with 10 cc. more. At the end of an hour the excess of "free" acid was found by 0.1 *N* alkali and methyl orange.

TABLE XV.—COMBINATION OF HCl, HBr AND HI WITH 750 MG. OF PROTEINS AS DESCRIBED.

	Cc. 0.2 *N* HCl.	Cc. 0.2 *N* HBr.	Cc. 0.2 *N* HI.
Egg	3.4	3.0	3.8
Fibrin	3.5	3.7	4.5
Casein	2.2	2.1	1.5

In each case the fibrin seems to hold more acid and the casein less than the egg. We should expect the same volume of acid to be combined with the different proteins. The discrepancy is probably due to the lack in delicacy in the behavior of this indicator in presence of undigested protein, but the results are close enough to show that the combinations are of the same type.

[1] THIS JOURNAL, 37, 1333 (1915).

In place of titrating directly, similar mixtures were filtered and the residues on the filter washed with successive equal portions of water, 20 cc. in each case. These washings were titrated separately. It was found that the fibrin held the acids more tenaciously than the other proteins. In seven or eight washings about one-fifth of the hydrochloric acid could be removed from the egg, a tenth from the fibrin, and a fourth, or less, from the casein. The dissociation of the hydrobromic acid is of the same order.

The behavior of hydriodic acid is most interesting here in view of the large weight held after evaporation experiments. The figures of Table XVI show the amount of 0.2 *N* NaOH required to titrate the first filtrate and successive washings in experiments with egg and fibrin.

TABLE XVI.

	Egg.		Fibrin.	
	a.	*b.*	*a.*	*b.*
1st filtrate and washing.....	13.75 cc.	14.30	12.55	13.20
2nd filtrate and washing.....	1.90	2.25	1.55	1.90
3rd filtrate and washing.....	1.25	1.30	1.50	1.20
4th filtrate and washing.....	0.90	0.60	0.70	0.90
5th filtrate and washing.....	0.60	0.20	0.60	0.40
6th filtrate and washing.....	0.30	0.00	0.50	0.20
7th filtrate and washing.....	..	..	0.40	0.10
8th filtrate and washing.....	..	..	0.20	..
9th filtrate and washing.....	..	..	0.10	..
Total.....	18.70	18.65	18.10	17.90
Cc. 0.2 *N* HI held.....	1.30	1.35	1.90	2.10

It is seen that after the prolonged washings about two-thirds of the HI held by the egg is washed out and over half of that held by the fibrin, as measured by the direct titrations. While the procedure is lacking in quantitative accuracy it gives a good comparative result. It is evident that when heat is not applied the hydriodic acid is very loosely held. The type of salt produced in this way suffers extended dissociation by water.

Resume.

The experiments detailed above show that the amounts of the halogen acids which combine with casein, fibrin and egg albumin, as measured by the usual indicator titration, are low and not accurately proportional to the molecular weights of the acids: The discrepancies are probably due to the lack of delicacy of the indicator in presence of unchanged protein, on the one hand, and to the more or less complete dissociation of the protein-acid compound on the other. The latter is doubtless the more important factor, since it has been shown that a large fraction of the acid may be washed away from the protein.

When the proteins named are treated with the halogen acids of 0.2 *N* concentration, in excess, and the mixtures evaporated at a low temperature by standing over sulfuric acid some weeks, followed by similar treatment over solid alkali, and final drying to constant weight at 75°, very constant weights of acid are taken up and held by the protein. These weights of acid are not increased by the excess added, which points to the definite character of the reaction. The amounts are not proportional to the molecular weights of the acids, the combining proportion being relatively greater for HI than for HBr and greater for the latter than for HCl. But the compounds all appear to be salts of the protein molecule and contain many times as much acid as is suggested by the titration combinations. These dry salts undergo dissociation readily when mixed with water.

If the acid-protein mixtures are evaporated on the water bath, in place of being dried at a low temperature, the behavior of HCl and HBr remains essentially the same. No greater amounts of the acid are taken up by a gram of protein and we doubtless reach here a maximum in the combining power of the acid and protein. A salt of a type different from that formed in solution at a low temperature is secured. In the case of the HI, however, there is no such limit to the iodine held, and it is probable that we have here a substitution of the element in the nucleus of the protein molecule, as well as an addition of the acid. As much as 75% of the weight of the original protein may be so held and the combination has a brownish ocher color, with loss of protein reactions.

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STUDIES ON THE CULTURE MEDIA EMPLOYED FOR THE BACTERIOLOGICAL EXAMINATION OF WATER.

I. THE SCHARDINGER-DUNHAM MEDIUM FOR TESTING FOR THE PRESENCE OF HYDROGEN SULFIDE FORMING BACTERIA.[1]

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Received March 27, 1915.

Introduction.

Although an enormous amount of time and labor has been spent upon investigations of bacterial culture media with a view of shortening the time required for diagnostic results and producing media yielding more uniform and more constant results, a critical review of the literature fails to show, save in a few instances, that the media which have eventually been proposed have received truly systematic study and that the concentrations suggested are necessarily those which are best fitted for the purposes for which the media have been made.

[1] Papers read at the Rochester Meeting, American Chemical Society, September, 1913.