On the gastric digestion of proteids / by A. Lockhart Gillespie.

ON THE GASTRIC DIGESTION OF PROTEIDS.

By A. Lockhart Gillespie, M.D., F.R.C.P.E., *Medical Registrar, Edinburgh Royal Infirmary.*

The following paper has been partly extracted from a thesis presented to the University of Edinburgh for the degree of M.D. in August 1892, and for which a gold medal was awarded to the author. A summary of the results was read before the Biological Section of the British Association for the Advancement of Science (Edinburgh meeting) in the same month, of which very condensed abstracts were printed in the *Proceedings* of the Association and in the *Medical Press and Circular.* The experiments on which the observations rest were performed in the Laboratory of the Royal College of Physicians, Edinburgh, and to the Laboratory Committee of the College my best thanks are due for the liberality displayed to me, and particularly to the Superintendent, Dr Noël Paton, for his ready help when I happened to be in difficulties.

My object throughout has been to endeavour to elucidate some of the problems presented by the gastric digestion of proteids. A great part, therefore, of my work has been directed towards the relations between hydrochloric acid and proteid substances.

That some kind of combination could exist between this acid and proteids has been known for some time. Danilewsky,[1] Richet,[2] Leo,[3] and Ewald[4] have demonstrated its existence, and more lately Hayem[5] and Winter have placed the subject on a more satisfactory footing.

I have elsewhere[6] called the combinations between HCl and proteids Proteid-hydrochlorides, and this term I shall continue to use throughout this paper.

[1] *Centralblatt für med. Wissenschaft*, 1888.
[2] Sur le suc gastrique chez l'homme et les animaux, 1878.
[3] *Centralblatt für med. Wissenschaft*, 1889.
[4] *Virchow Archiv*, xc., ci., civ.
[5] Du chimisme Stomacale, 1891.
[6] *Medical Press and Circular*, August 1892.

Methods.

I must in the first place briefly note the methods I employed in my experiments. The total acidity of any solution was estimated in the ordinary way by means of a decinormal solution of NaOH; and an alcoholic solution of phenolphthalein. The results were all calculated out in terms of HCl. The amount of the combined acidity in a specimen was arrived at by estimating its acidity before and after evaporation at 100° Cent. If an aqueous solution of HCl be evaporated in an evaporating dish for a sufficient time none remains, but if albumen be added to it beforehand, no matter how long the heat be applied, some hydrochloric acid remains behind. Hayem and Winter's method of applying this fact to the determination of the different forms of acidity is more accurate than mine, but much more elaborate. They worked with chlorine alone, they estimated the total chlorine by means of chromate of potash and silver nitrate, after adding to the solution to be tested carbonate of soda, to transform all the chlorine into NaCl, and by fusing this, to burn off the proteids. The chlorine combined with proteids was calculated by a similar procedure after the solution had been dried at 100° for some hours, the chlorine originally fixed to bases, &c., having been calculated beforehand, by simple incineration of another quantity of the fluid, and then subtracted. The chlorine combined with proteids and the fixed chlorine together when subtracted from the total chlorine, gave the free chlorine or HCl. In the following pages, when the chlorine was estimated, this process was made use of. It is, however, long, troublesome, and in the many incinerations a small quantity of chlorine is undoubtedly lost. If carefully done it is suitable for laboratory work; for practice it is too cumbersome. Simple estimation of the acidity before and after evaporation for some hours at 100° or 110° is, to my mind, sufficient for most practical purposes.

The different proteids were separated from each other in the usual way. The albumoses from peptone by saturation with ammonium sulphate when warm, after the addition of a small quantity of acetic acid. The different albumoses from one another by saturation with NaCl and dialysis. Albumen was procured almost pure from white of egg, the globulin being first precipitated by copious dilution with distilled water, the salts in some cases dialysed off, and the solution dried at 40° Cent. in large open dishes. The fibrous tissue had been previously got rid of by shaking up the white of egg with broken glass and filtering.

Other matters regarding methods used are explained in the text.

The Functions of the Stomach.

Czerny's dog lived for several years without a stomach, and to all appearance suffered little inconvenience. Personally, I should not like to do without this organ, but I have little

doubt that if I fed as moderately and as simply as a laboratory dog, I would suffer little from the want. As it was proved, therefore, that the stomach was not indispensable to the organism as an agent for digesting food, an idea which was long and widely entertained, some other theory had to be framed to account for its rôle in the animal economy. It was allotted purely an antiseptic function—it was styled the Cerberus of the digestive tract: past it, in health, only the most resistive and least poisonous organisms could pass. Against this view, however, is the fact that Nature or Evolution has given the stomach a reagent which is largely antagonised in its antiseptic action by that very kind of food which it is mainly capable of digesting; in other words, proteids act as Cerberus's sop. Another function which may be attributed to the stomach is the bringing of foods to a proper consistency before ejecting them into the duodenum, excess of fluid is absorbed, deficiency made good. Witness the state of the gastric contents after a meal of pure porridge, with no fluid added,—in a short time the contents are much diluted; while, on the other hand, if a large quantity of fluid be taken as well, the consistence of the food at the end of a similar time is almost identical.

The stomach is useful also in mixing and subdividing the food, and thus, in allowing no large and irritating masses to enter the duodenum, it allows animals to take a large meal without discomfort; for without such a receptacle, men and animals would always have to be eating, leaving no time for other pursuits. Lastly, the amylolytic action of the saliva is checked. The attempted allocation of one supreme function to the stomach is, I believe, foredoomed to failure. It can do many things well, or perhaps I might say, fairly well, a wise instance surely of the law of conservation of energy so often manifested elsewhere in nature.

The Affinity of Proteids for Hydrochloric Acid.

If some albumen—it matters not whether serum or egg albumen—be dissolved in water, the solution placed inside a parchment tube, and the tube immersed in a solution of hydrochloric acid in water, in time, after a few hours, the fluid inside

will be found to be more acid than that outside. That this is not due simply to the higher specific gravity of the fluid inside, is shown by the fact that if a solution of sugar or other carbohydrate be substituted for that of the proteid, the acidities in and out become equal. Any other proteid has the same effect as albumen. HCl, then, has an affinity for proteids.

Now, if the tube containing the albumen and acid be removed from the acid solution and placed in a beaker containing water, much of the acid dialyses out again, and in time, if the water be changed often enough, all may be removed. The affinity of the acid for proteids is weak, and may be overcome by the action of dialysis into pure water. This is shown by the following experiment.

Experiment I.

A neutral solution of proteids of unknown strength was placed inside the tube of a dialyser, the tube immersed in a ·612 per cent. solution of HCl; in two and a half hours the acidity inside was ·432 per cent. and in twenty-four hours ·684 per cent. Distilled water was then substituted for the hydrochloric acid outside; in two and a half hours the acidity of the proteid solution was ·468 per cent., and in twenty-four hours ·09 per cent. That is to say, that most of the acid had dialysed out, but a residue remained. This residue gave none of the tests for free HCl.

On subjecting this to the further action of dialysis into distilled water all traces of acidity disappeared in forty-eight hours.

Again, if the acid be in large proportion as compared with the proteid, and the contents of the tube are analysed after dialysis of the acid to the inside, we find the acid to be in two forms—acid which is free and acid which is organically combined. On estimation of the free acidity also, less free acid is found inside than there had been outside before removal of the parchment tube with its contents, but the sum of the combined and free acidity inside always exceeds the total acidity outside. That is to say, that HCl organically combined, even if it be in excess, does not hinder the further dialysis of free HCl. This point is of importance in artificial gastric digestion by means of a dialyser, and also in natural digestion. It is clearly shown in the following dialyser experiments.

Experiment II.

A solution containing beef bouillon, gelatine, and egg albumen

was placed inside a parchment tube, and this suspended in a ·036 per cent. solution of HCl. In two and a quarter hours the solution inside, which had been almost neutral, had an acidity of ·324 per cent., or nearly ten times that out, of this only ·027 per cent. was free.

Experiment III.

In this the fluid inside was a little more dilute; and although the solution outside was as high as ·198 per cent. in acidity, the acidity inside never rose above ·288 per cent., or less than in the last experiment when the acidity outside was only ·036 per cent. When the acidity in this experiment was at its height only ·09 per cent. was free.

Properties of a Proteid-hydrochloride.

If a certain quantity of hydrochloric acid, say 5 grammes, be added to 100 grammes of egg albumen, and the resulting mixture tested, no free HCl can be found. That is to say, a drop of the resulting solution strikes no scarlet colour with Günzberg's vanillin-phloroglucin, with Boas' resorcin, with Möhr's sulphocyanide reagent. On testing it by Sjoquist's process, or by Leo's or Mintz', no free HCl is shown. And again, on drying some of the solution on a water bath for a couple of hours or so, no change in acidity occurs on redissolving the dry residue in distilled water and titrating with soda. On the other hand, if a simple solution of HCl in water be evaporated for the same length of time, no trace of acid is left.

This combination of hydrochloric acid with albumen is therefore not destroyed by a heat of 100° Cent., and does not give any reaction when treated with the aniline reagents commonly used for the identification of free mineral acids. It has, however, the same or almost the same acid value as the original HCl; or to put it otherwise, HCl combined to proteids neutralises the same quantity of decinormal soda solution as it does when it is in a free state.

Experiment IV.

3 ccms. of a solution of egg albumen (·3465 grm.) were added to 15 ccms. of a ·2304 per cent. HCl solution (·0345·6 grm.).

The mixture was tested with Günzberg's reagent, and no free HCl found. That is, 9·98 per cent. of acid had combined with the albumen; the acidity, however, was ·192 per cent., or equal to that before.

1 ccm. more was added (·002304 grm.); free HCl was present with 10·6 per cent. of acid.

Experimemt V. Performed in the same manner.

1.	2 ccms.	albumen	solution	= ·23	gramme	+ 13	ccs.	·18	per cent.	HCl.
2.	2 ,,	,,	,,	= ·23	,,	+ 7	,,	·342	,,	HCl.
3.	2 ,,	,,	,,	= ·23	,,	+ 50	,,	·045	,,	HCl.
4.	5 ,,	,,	,,	= ·575	,,	+ 9	,,	·342	,,	HCl.

The results were :—

1. 10·17 per cent. of acid, free HCl present.
2. 10·4 ,, ,, ,,
3. 9·18 ,, free HCl absent.
4. 5·34 ,, ,, ,,

Acidity of Nos. 3 and 4 were ·044 per cent. and ·219 per cent. respectively.

It is more difficult to arrive at the exact amounts of each when both free and combined hydrochloric acid are present in the same solution. Drying a few cubic centimetres at 100° Cent. drives off the free HCl; but the charring of the proteid, and the resolution of some of it into simpler forms, one of which has a violet colour (Liebermann's reaction for free HCl), tend to vitiate the results. Adding, drop by drop, a decinormal solution of sodium hydrate until the fluid tested ceases to give any reaction with vanillin-phloroglucin is also fallacious, as some of the combined acidity is affected as well as, and at the same time as, the free. Neutralisation of the free HCl with carbonate of barium, which is said not to affect the combined acid, gives better results, but the process is tedious. On the whole, I prefer drying the fluid at 100° and then titrating again: the difference between the total acidity and the acidity left after drying gives the free HCl, and the fallacy present is constant and very small, the free acidity in fact being always slightly too great, the combined slightly too small.

Although this combined acid is equal quantitatively to the original free HCl, it has not retained any of the other properties of the free acid in the same proportions. For, as I have said above, it does not give any of the common reactions for the free acids, but on investigating its action further I find that its antiseptic property is very slight. I have grown the *Staphylococcus pyogenes aureus* in a ·68 per cent. solution of combined HCl,[1] while the amylolytic action of saliva goes on in a solution of combined acidity double the strength of that necessary, if the acids were free, to destroy the ferment.

[1] *Journal of Pathology*, 1892.

This last fact is of interest with regard to the previous work which has been done on the subject. For instance, Chittenden and Ely[1] found that ·05 per cent. HCl if added to saliva rather increased its action on starch. Astachewsky[2] affirms the same. Langley[3] worked at the subject and found the following facts :—

Ptyalin is destroyed by solution of HCl above ·005 per cent.
,, ,, $NaCO_3$ above ·5 ,,

—while the same author, when working with Eves,[4] found that ·0015 per cent. HCl distinctly diminishes the action : he got best results from saliva which was absolutely neutral.

Experiment VI.

Some starch paste was made of a strength of 1·5 per cent. I collected 15 ccms. of my own saliva and filtered it. The alkalinity was not tested. A solution of proteid hydrochlorides was also made by addition of proteid material, albumen, and albumoses to a solution of HCl until no free HCl remained. The acidity of this solution was ·504 per cent.

The details are as follows :—In seven flasks were placed—

5 cc. of starch and 1 cc. of saliva +	2 ccs.	HCl ·504 per cent.	combined.
	2 ,,	HCl ·252 ,,	,,
	2 ,,	HCl ·144 ,,	free.
	2 ,,	HCl ·072 ,,	,,
	2 ,,	HCl ·126 ,,	combined.
	2 ,,	HCl ·063 ,,	,,
	2 ,,	water.	

That is to say, that there was in the flasks—

1.	·01008 gr.	HCl or	·126	per cent.	all combined.
2.	·00584 ,,	HCl ,,	·063	,,	,,
3.	·00288 ,,	HCl ,,	·036	,,	mostly free.
4.	·00144 ,,	HCl ,,	·018	,,	,,
5.	·00252 ,,	HCl ,,	·0318	,,	all combined.
6.	·00126 ,,	HCl ,,	·01575	,,	,,
7.	·00000 ,,	HCl ,,	·0		

The flasks were put in a hot air chamber and kept at 38° Cent. for half an hour. The contents of each was then tested with Fehling's solution, and with iodine and iodide of potash.

1.	No sugar,	Blue with iodine.
2.	,,	,,
3.	,,	,,
4.	,,	,,
5.	Trace of sugar,	Slight violet tinge.
6.	Sugar present,	Decided violet.
7.	,,	Red violet.

[1] *Journal of Physiology*, vol. iii., and in *American Chem. Journal*, vol. iii. 305.
[2] *Centralblatt für med. Wissenschaft*, 1878.
[3] *Journal of Physiology*, iii. p. 246.
[4] *Journal of Physiology*, iv. p. 18.

It is clear, then, that although the addition of ·018 per cent. of free HCl was sufficient to stop the amylolytic action of the salivary ferment, ·0318 per cent. of the combined acid only checked it, while ·01575 per cent. had little effect.

As the hydrochloric acid in the stomach at the commencement of digestion of a proteid meal is entirely combined, it is obvious that the continuation of the amylolytic action of the salivary ferment is possible for a short time.

Forms of Proteid-hydrochloride.

All the reactions mentioned above may occur in the absence of pepsine, when acid and proteid are simply brought together. The amount of acid taken up by the albumen under these circumstances is nearly constant,—some variation, however, being observed in direct proportion to the strength of acid solution used. Without pepsine, hydrochloric acid combines with albumen only up to the proportion required to form acid albumen: the proportion may be less than that required, but never more, unless we look on the charring effect of strong acid as primarily a combination. This acid, nevertheless, though now combined, is still able to act further on the proteid if pepsine be added to the solution. For, if pepsine be added to a mixture of HCl and albumen, part of which is acid albumen and part serum albumen, but in which there is no free HCl present, further and simpler proteid bodies are formed, but only to a limited extent, and then at the expense of some of the acid albumen, which perhaps may become serum albumen again.

Hydrochloric acid combining with proteids in the absence of pepsine can saturate the molecules, but cannot split them further.

Experiment VII. illustrates this fact, and will also serve as a text for further observations on the action of HCl on proteids in the presence of pepsine.

A solution of egg albumen, of which 9 ccs. contained 1 grm., was used, to which solution hydrochloric acid in varying strengths was added. All the figures in the tables have been corrected for ash.

The quantities were as follows :—

1.	2	grms. albumen	·38528	grm. HCl	86	ccs. in all,	·448	per cent. acidity.
2.	1·98	,,	·24948	,,	66	,,	·378	,,
3.	1·96	,,	·08424	,,	36	,,	·234	,,
4.	1·96	,,	·08424	,,	36	,,	·234	,,

After three hours at 38° Cent. the condition of the acid was tested with regard to whether it was present in the free state or not.

Condition of the hydrochloric acid in the above fluids.

No.	Total Acidity per cent.	Combined Acidity		Free Acidity		Liebermann's Reaction.
		per cent.	HCl in grms.	per cent.	HCl in grms.	
1	·448	·27	·2322	·178	·15308	+
2	·378	·252	·15448	·126	·095	+
3	·234	·234	·08424	...	...	...
4	·234	·234	·08424	...	...	...

Percentages of combined HCl to proteid present.

No.	Total Proteid in grms.	Combined Acidity in grms.	Percentage.
1	2	·2322	11·61
2	1·98	·15448	7·8
3	1·96	·08424	4·29
4	1·96	·08424	4·29

The exact amounts of the solutions figured in the first of these tables were now poured into dialyser tubes, and 150 ccms. of distilled water placed round each.

To Nos. 2 and 3 ·054 grm. of pepsine was added.

The four beakers with their contents were now placed in an incubator at 38° Cent. In six hours the amounts of the proteids and the acidities were again estimated.

Acidities after six hours' dialysis.

a. Inside.

No.	Amount in ccms.	Total Acidity		Combined Acidity		Free HCl		Liebermann's Reaction.
		per cent.	in grms.	per cent.	in grms.	per cent.	in grms.	
1	91	·216	·19656	·198	·18018	·018	·01638	+
2	75	·2052	·1539	·2052	·1539	...	...	...
3	50	·126	·063	·126	·063	...	...	...
4	46	·144	·06624	·144	·06624	...	...	...
	b. Outside.							
1	141	·108	·1522	·02119	·029888	·0868	·1224	+
2	130	·082	·1066	·07759	·10087	·00441	·00573	+
3	130	·216	·02808	·0216	·02808	...	...	...
4	136	·0072	·009792	·002104	·002862	·00693	·00942	+

Proteids after six hours' dialysis.

a. Inside.

No.	Albumen.	Acid Albumen.	Albumose.	Peptones and Extractives.	Total.
1	·0	1·8564	trace	·02073	1·87713
2	·12	·245	·28	·8036	1·4486
3	·851	·42	·101	·29922	1·67122
4	1·0948	·7314	trace	·0404	1·8666
	b. Outside.				
1	...	...	trace	·12287	·12287
2	...	...	·076	·4754	·5514
3	...	...	·018	·291876	·309876
4	...	...	trace	·0934	·0934

Total proteids or organic solids recovered in grams.

No.	Inside.	Outside.	Total.
1	1·87713	·12287	2
2	1·4486	·5514	1·99
3	1·67122	·309876	1·981096
4	1·8666	·0934	1·96

Total solids and ash found outside.

No.	Total Solids.	Ash.	Organic Solids.
1	·196698	·073814	·122884
2	·63	·0786	·5514
3	·38369	·073814	·309876
4	·1672	·0738	·0934

This is a long and seemingly complicated experiment. Careful study, however, soon unravels its intricacies.

The result of No. 1 is as follows: originally albumen with excess of HCl, finally acid albumen with a small quantity of diffusible organic material and still an excess of free acid.

In No. 2 there was also originally an excess of HCl, amounting, however, to only ·095; judging from the previous example, however, as there was some free acid present, all the albumen would be in the form of acid albumen. On the addition of

pepsine, there is a marked change in the results, no free acid inside, only a trace outside, while some of the albumen can be recovered unchanged. The other varieties of proteids are also present, with an appreciable amount of albumose (deutero) outside. At the end of the experiment only ·0053 grm. of free HCl was available, at the beginning there was ·095: ·0897 gr., therefore, have been required to perform the work indicated by the amounts of the lower proteids.

In No. 3, although the HCl added at the beginning was all combined to proteids, the addition of pepsine to this enabled a further digestion. The dialysis, though, of peptones prevented the presence of free acid outside.

In No. 4, under the same circumstances, but without the presence of pepsine, a small quantity of organic material was lost in some way, but the proportion of acid albumen to albumen is instructive, as indicative of the energy of HCl. The presence of free HCl outside is explained by the power which simple dialysis has of separating HCl from proteids.

If the details of the experiment be more broadly considered, it will be seen that in Nos. 1 and 2 the diminution of free HCl at the end of the experiment was ·0143 gr. and ·0897 gr. respectively, the sole difference in the working being the addition of pepsine. The decrease in free HCl is proportionate to the increase of simpler proteid forms. Throughout Nos. 3 and 4 digestion has gone on in the absence of free HCl, and it also follows that the addition of a very small quantity of free HCl and a little pepsine to a mixture of albumen and acid albumen, which contains no free acid, results not in more acid albumen, but in the splitting up of this proteid into peptones and albumoses. The amount of albumen remains the same, or even increases. This is also seen in the case of No. 2, where, although all the albumen had probably been converted into acid albumen before the pepsine was added, yet after further digestion some albumen could again be separated.

On the other hand, HCl combined with proteids during an act of digestion, and therefore in the presence of pepsine, is incapable of further action; and if all the acid be so combined, digestion will not proceed until further free acid be added. In the combination of the acid with the proteid without pepsine,

potential energy is stored up ready to split up the molecules of albumen or albumose—for HCl combines with all proteids in a similar way—when further action is facilitated by the addition of pepsine.

For it is not to albumen alone that HCl unites. If to a solution of proto-albumose hydrochloric acid be added, a compound with or a hydrochloride of proto-albumose is formed, but it is not until pepsine is added that this albumose can be changed into deutero-albumose or into peptone. Hydrochloric acid is able in the presence of pepsine to over-saturate and thereby to split up proteid molecules.

Mode of Digestion of Proteids.

These observations suggest a simple way of explaining the course of the gastric digestion of proteids. Suppose that I have, to begin with, some serum albumen in solution in the tube of a dialyser, to which I add a few grains of pepsine and surround it with a weak solution of hydrochloric acid, the whole apparatus being kept at 40° Cent. As the molecules of the acid dialyse through the membrane they combine with molecules of the albumen. The albumen molecules are so large that in all probability more than one molecule of HCl is required to unite with each to form acid albumen. There may be, therefore, an intermediate stage, in which acid is combined to albumen, but no acid albumen has yet been formed. When some of the albumen molecules have been transformed into acid albumen, and while free HCl is still dialysing through the membrane, more acid combines with the acid albumen, and in the presence of pepsine this combination affords sufficient energy to split up the larger molecule into two smaller ones, each of which can unite with a larger proportion of acid than its parent. We have now got to proto-albumose, and the same applies to the splitting of this into deutero, and of deutero albumose into peptone.

How does this theory accord with fact? Fairly well. Previous observers have investigated the subject.

Danilewski[1] was the first who tried to estimate the exact amount of acid which combined with proteids, coming to the conclusion that

[1] *Centralblatt für med. Wissenschaft*, 1888.

10 to 12 parts of HCl were taken up by 100 parts of proteid material. Ewald[1] supported his conclusion, while Herth,[2] working in Maly's laboratory, found that the hemialbumoses took up HCl with avidity. Moritz[3] gives 8 to 12 parts of albuminates to 1 part of HCl. Pfungen,[4] testing with methyl violet (a very uncertain reagent), notes that no free HCl appeared until after ·843 grm. of HCl had been added to 100 grms. of beef, ·674 grm. of HCl to 100 grms. of bread, and ·504 grm. of HCl to 100 ccms. of milk. Sansoni and Molinari,[5] finally, in a very interesting research, have tried to solve this problem. They added to a known quantity of a decinormal or a centinormal solution of HCl a solution of an albuminoid until the fluid ceased to give a reaction with phloroglucin-vanillin. They note that soluble proteids take up the acid quickly, the insoluble slowly.

My results are obtained by the evaporation of mixtures of proteids and hydrochloric acid at different temperatures. The following table gives the sum of a number of experiments.

Proteid.	HCl Per Cent.			
	Without Pepsine.		With Pepsine.	
	Evaporation at 100° C.	Evaporation at 38° C.	Evaporation at 100° C.	
Egg albumen, . .	7·2	14·45	19·8	18·8
Globulin, . . .	8·6	15·97	17·1	19·8
Acid albumen, . .	9	18·4	18·4	18·8
Proto albumose, .	11	23·6	18	19·8
Deutero albumose, .	14	27·9	18	20·2
Peptone, . .	18–20	31·95	19·4	21

The strength of the HCl solution used in these experiments was ·27 per cent. Evaporation was continued for two hours after the contents of the capsules were dry. In each case excess of acid was added.

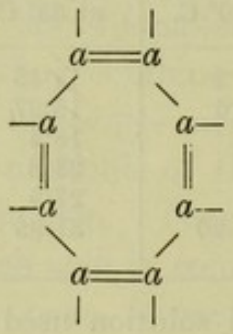
It is at once clear that evaporation at 38° Cent. is insufficient to drive off all the free HCl, and it is as apparent that there is a great difference between those in which pepsine had been added previously and the specimens evaporated without previous digestion. The amount of acid combining in the series in which this digestion had occurred was much the same throughout.

[1] *Die Klinik der Verdauungs Krankheiten*, Berlin, 1889.
[2] *Monatschrift für Chemie*, 1884, S. 206.
[3] *Deutsch. Archiv für Klin. Med.*, xliv.
[4] *Wiener Klin. Wochenschrift*, vi.–x., 1889.
[5] *Annali di Chimica*, ix., 1889, pp. 13 and 329.

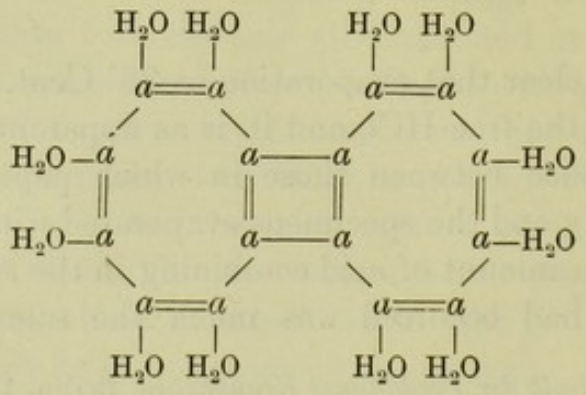
In the other cases the amount of acid per cent. increased as the proteids became simpler. The totals in the last two columns closely approximate to the total in the simplest proteid in the first series.

The Probable Relation of the Proteid Molecules to one another.

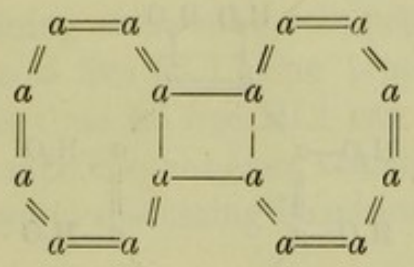
Speculating on these *data* I have been led to project a scheme illustrating the splitting up of the albumen molecule. I must first premise that in using the number 8 or multiples of 8, I simply consulted my own convenience. And again, that I have used A = A to signify the primary proteid molecule, here again using a double form to simplify matters. As the benzine nucleus is represented in chemistry as  —so, for illustrative purposes, I will use this form as a graphic representation of my scheme :—



Serum-albumen I would represent thus :—

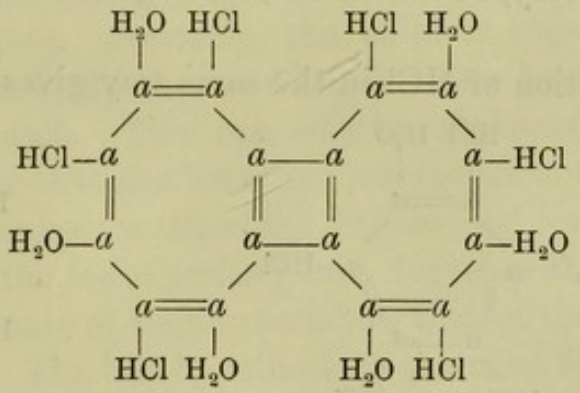


or $8A_2+12H_2O$. This is the constitution of it when uncoagulated or when dissolved in water. If coagulated by boiling, the form changes to 16A.



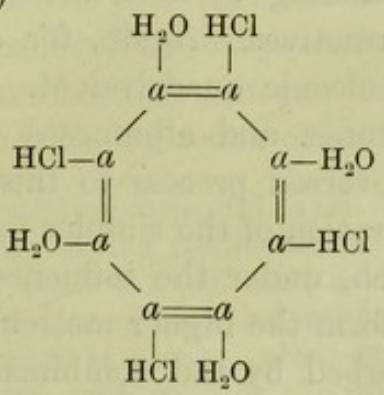
In this form it is insoluble in water, and more difficult to digest than in the former.

Acid albumen has almost the same properties as serum-albumen: it is indiffusible, is coagulated on boiling under certain circumstances, and readily returns to the form of albumen: it probably has nearly the same size of molecule in it: six

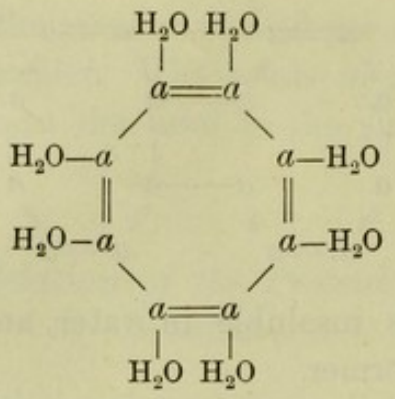


molecules of water are replaced by six molecules of HCl, or the formula is $8A_2 + 6H_2O + 6HCl$.

Leaving hetero-albumose out of consideration for the present, proto-albumose has clearly a smaller molecule than the preceding; it is not coagulated by boiling, and it dialyses to quite an appreciable extent. In the presence of pepsine and free HCl another molecule of the latter combines with an A_2, and in so doing splits up the acid-albumen molecule into $(4A_2 + 4H_2O + 4HCl)$

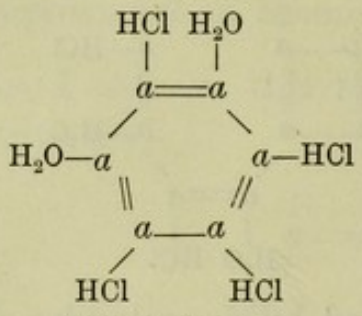


this when washed gives pure proto-albumose.

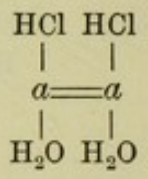


I do not see why hetero-albumose may not be looked upon as the preceding without the water molecules; at anyrate it is easily formed from proto-albumose, and in this form would be analogous in theory, as it is in fact, to coagulated albumen, insoluble in water.

Further addition of HCl in the same way gives



Deutero-albumose.



Peptone.

That deutero- has a smaller molecule than proto-albumose is proved, I believe, by the fact that it dialyses more quickly, and the same argument applies to peptone.

In this very hypothetical scheme, I have looked on the primary and indivisible proteid molecule as $\overset{|}{A}=\overset{|}{A}$, and postulate the inability of A standing by itself without splitting up into amido-acids and extractives. Again, for convenience, I have made each simple molecule quadrivalent. The building up of albumen from peptones and albumoses in the walls of the stomach may be a reversed process to this, probably consisting in the partial dehydration of the simpler forms, leaving some of the links open, which, under the influence of a ferment, clasp one another and re-form the higher molecule.

That HCl is absorbed by and combined to proteids during digestion is also proved conclusively by the fact that if a mix-

ture be made containing some albumen, pepsine and HCl, only a very small amount of free HCl being present, after digestion has gone on for some time no free HCl can be detected. The total acidity of the mixture, however, remains the same. This was referred to before in discussing Experiment VII.

Size of the different Proteid Molecules.

The different arguments in favour of the lower proteids being simpler, the higher more complicated molecules may now be brought together in one place. *First,* the lower forms can be obtained from the higher, and contain the same elements in very much the same proportions. This has been shown by various analyses. *Secondly,* the sulphur constituent of each proteid is practically the same—somewhere between one or two molecules in each. This can only be explained on two hypotheses—either that the lower are rearrangements of the higher proteid molecules, or that the higher are formed of several molecules of the lower series joined together in some way, each primary molecule of which resembles that of the lowest proteid in the scale. The last hypothesis is the most feasible and most workable. *Thirdly,* differences exist in the proportion of different metals combining with different proteids.

Silver Salts of the Proteids.

In pursuit of information regarding the relative sizes of the proteid molecules, I was led to make researches into the bodies which are formed when silver nitrate is added to them. The actual working out of the problem I must leave for another occasion. I only wish to give here the proportionate weights of the bodies formed under identically similar circumstances. The results obtained have been so uniform, that I have ventured to call the combinations by the name of salts.

Loew (*Archiv für Physiologie*, Bd. xxxi. s. 393), who worked at this subject, obtained varying results as he varied the strengths of his silver solution : below a 1 per cent. he got no precipitate with egg-albumen without the addition of sulphuric acid, with a 1 per cent. he found the ratio of silver to albumen as 2·18 per cent.; with a 5 per cent. to a 10 per cent. solution the ratio rose to 4·31 per cent. Further manipulation afforded combinations richer in silver,—the

addition of ammonia, for instance, causing the precipitate to contain 10·7 per cent. of silver.

Loew estimated the amount of the silver by dissolving the ash of the washed precipitate in nitric acid, precipitating with hydrochloric acid, and weighing as chloride of silver. He considered that atoms of metallic silver were attached directly to the proteid molecule.

Fuchs (*Ann. Chem. Pharm.*, Bd. 151, p. 372) found in his silver albuminate an average of 3·28 per cent., but he always had an excess of albumen present.

Loew also investigated the amount of silver which combined with "peptone," but in those days "peptone" comprised albumoses as well as true peptone. He only made three analyses with a mean of 11 per cent. of silver. All through his work this observer has theorised from too scanty grounds, two or at most three analyses sufficing in each case.

As the percentage of silver to albumen varied so much in these results, it was doubtful whether the bodies obtained were true chemical combinations or not. But it seems likely that a molecule of silver may take the place of one molecule of water, or that two or more may be so added to the albumen. Loew's figures are also borne out by Harnack's copper compounds. This observer succeeded in separating two series of cupric-albuminates, which correspond closely to the first two silver compounds of Loew.

I have made an extended series of observations on the silver salts of the proteids. For this purpose I separated the globulin from white of egg as described above (p. 196), neglecting however the salts. These, however, I estimated in the usual way, calculating also the weight of chlorine by precipitation of the dissolved ash with nitrate of silver. I adopted the same procedure with regard to the other members of the proteid group. In the case of deutero-albumose and peptone, I found that so much of the proteid was lost during the dialyses necessary to abstracting the salts, that it was better to calculate the ash and chlorine as above stated. All the specimens of individual proteids, however, were free from traces even of the others.

I added to each proteid in turn an excess of silver nitrate, dissolved in distilled water, the solutions being of unknown strength, but usually above 10 per cent. The precipitate with albumen was pure white, flocculent and comparatively light, the solution filtering slowly. With proto-albumose the precipitate was finer, of a bright light-yellow colour and filtered well. Deutero-albumose, on the other hand, gave an orange yellow, fine, heavy precipitate, filtering rapidly, while peptone afforded a slightly yellow, very minute precipitate, which filtered badly, much going through the filter-paper at first, and which washed extremely ill. The precipitates were washed with distilled water until no further reaction was given

on the addition of HCl to the filtrate or precipitate caused by the addition of alcohol. They were then washed with methylated spirits and absolute alcohol, dried at 110° and weighed. The filter-papers used were practically ash free, each containing ·000085 grm. The weighed paper was now incinerated in a capsule of known weight, the silver recovered and estimated. On occasion the result was checked by solution of the silver in HNO_3, filtering, then precipitation with HCl and reweighing.

The silver obtained by the incineration was both in the form of chloride, from the chlorine in the ash, and of the metal, the oxide Ag_2O and carbonate being converted to the metal by the prolonged action of heat. The chloride of silver in the remainder had already been calculated from the known chlorine of the ash of the original proteid, and could be subtracted.

The results are given both in percentages and in simple proportions.

Experiment VIII. A. *Silver to Egg Albumen.*

No.	Silver albumen.	Silver freed from chloride and ash of filter-paper.	Proportion of silver to albumen.	Percentage.
1	·191	·0129	1 to 14·8	6·75
2	·17	·0123	1 ,, 13·9	7·23
3	·16	·0106	1 ,, 15·09	6·62
4	·3972	·0277	1 ,, 14·33	6·97
5	·3371	·0242	1 ,, 13·92	7·22
6	·3961	·0261	1 ,, 15·1	6·58
7	·239	·0161	1 ,, 14·84	6·73
8	·246	·0167	1 ,, 14·73	6·78
9	·891	·0602	1 ,, 14·8	6·75

Average 1 to 14·61, or 6·847 per cent.

These numbers are very satisfactory when the fact is considered that the silver was added in varying strengths, though always in excess.

B. *Silver to Proto-Albumose.*

No.	Silver albumose.	Silver freed of chloride and of ash of filter-paper.	Proportion of silver to proto-albumose.	Percentage.
1	·081	·0136	1 to 5·94	16·79
2	·054	·0091	1 ,, 5·93	16·85
3	·065	·0105	1 ,, 6·19	16·15
4	·154	·0245	1 ,, 6·28	15·92
5	·07	·0112	1 ,, 6·24	16
6	·113	·025	1 ,, 6·01	16·63
7	·128	·02	1 ,, 6·4	15·62
8	·2722	·0468	1 ,, 5·81	17·2

Average figures, 1 to 6·1, or 16·395 per cent.

C. *Silver to Deutero-Albumose.*

No.	Silver albumose.	Silver freed of chloride and of ash of filter-paper.	Proportion of silver to deutero-albumose.	Percentage.
1	·092	·0217	1 to 4·239	23·58
2	·09	·0221	1 ,, 4·07	24·44
3	·075	·0169	1 ,, 4·43	22·57
4	·037	·0095	1 ,, 3·89	25·7
5	·04	·0102	1 ,, 3·92	25·5
6	·097	·0234	1 ,, 4·14	24·12

Average figures are 1 to 4·1149, or 24·318 per cent.

D. *Silver to Peptone.*

No.	Silver peptone.	Silver free from chloride and ash of filter-paper.	Proportion of silver to peptone.	Percentage.
1	·04	·1011	1 to ·39	256
2	·031	·0617	1 ,, ·502	199
3	·087	·275	1 ,, ·316	316
4	·098	·1141	1 ,, ·858	116

The average figures are 1 to ·5165, or 221·75 per cent.

It will be noticed at once that the numbers in the albumen series are more constant than in the proto-albumose compounds; the numbers in the deutero-albumose are still less constant, while little reliance can be placed on the peptone figures. The precipitate, in fact, of the peptone is probably very unstable.

From these figures the relation of the different proteids to one another is as follows:—

Albumen.	Proto-albumose.	Deutero-albumose.	Peptone.
14·61	6·1	4·1149	·5165 ?
or 1	·417	·281	·0353 ?

To check these results, I precipitated similar solutions of these bodies by means of a very dilute solution of silver nitrate, using ·1 per cent. in distilled water. This was added in excess. Very similar precipitates to those obtained before fell in the first three. With peptone, however, a precipitate appeared which was so diffuse and light that by no means could it be retained on a filter-paper. Two determinations were made in each case.

Experiment IX.

	Proteid and Silver.	Silver alone.	Proportion.	Percentage.
Albumen, . . . 1	·273	·038	1 to 7·18	13·9
,, . . . 2	·306	·04	1 ,, 7·65	13·07
Proto-albumose, . 1	·016	·0032	1 ,, 5	20
,, ,, . 2	0·155	·0027	1 ,, 5·94	16·79
Deutero-albumose, 1	0·22	·0067	1 ,, 3·28	30
,, ,, . 2	0·3	·0075	1 ,, 4	25

The combination with albumen here has double the quantity of silver in it; in the others also the silver is in slightly greater proportion. The numbers are too small to found any conclusion on, and require repetition.

The general result, then, of these observations is to point to the great probability of a descending series of proteid molecules. I attempted to check my results with a similar set of experiments on copper salts of the proteids, but the time at my disposal has proved insufficient.

Harnack (*Zeitschrift für Physiol. Chemie*, Bd. v. s. 198), who gives an account of the literature of the subject, found that egg albumen combined with 1·35 or with 2·64 per cent. of copper. He also submitted the bodies so obtained to analysis, and found the composition of the albumen was almost identical in the two.

The following figures, which, being derived from too scanty materials, are not to be relied on, corroborate to some extent the previous results.

Experiment X.

Copper sulphate solution was added in excess to the various proteids with the following result:—

Copper to Albumen, ... 1·5 per cent.
Copper to Proto-Albumose, . . . 2·9 ,,
Copper to Deutero-Albumose, . . . 4·17 ,,
Copper to Peptone. Could not be retained on a filter-paper.

As in the silver combinations, the albumen precipitate was flocculent, the others finer and heavier.

Diffusion of the different proteids, I thought, might also aid in determining the relative sizes, and the following series of observations was performed for that purpose.

Experiment XI.

The following amounts of the different proteids were each dissolved in 50 ccms. of distilled water, other 50 ccms. were placed outside the dialysing tubes, into which the proteid solutions were poured. The levels of the solutions inside and out were carefully rendered the same. After three hours the water outside was evaporated, the residue weighed in capsules. Corrections for ash were made in each case.

Inside.		Outside 50 ccs. water, which, after three hours, contained—
1. ·237 Egg albumen, .	+ 50 ccs. water.	1. ·0015 gr. or ·64 per cent.
2. ·24198 Proto-albumose, .		2. ·006 ,, or 2·47 ,,
3. ·24148 Deutero-albumose,		3. ·013 ,, or 5·38 ,,
4. ·0421 Peptone, . . .		4. ·01 ,, or 23·75 ,,

That is to say, that albumen dialyses little if at all, but that the others dialyse progressively faster as we go down the series.

The different facts which have been noted above about the relative size of the proteid molecules, and the consequent support they give to my theory of the combination of HCl with them in peptic digestion, may be here set down together:—

No.		Hydrochloric acid per cent.	Silver per cent.	Copper per cent.	Diffusion per cent.
1	Albumen, . .	7	6·847	1·5	·64
2	Acid albumen, .	9	...	...	...
3	Proto-albumose, .	11	16·395	2·9	2·47
4	Deutero-albumose,	14	24·318	4·17	5·38
5	Peptone, . .	20	221· (?)	...	23·75

All these series are of one form, though naturally, as the reagent varies, so the intervals vary. I do not propose to argue further from these figures on the present occasion.

Speaking broadly, then, I believe the class of proteids to consist of bodies which are multiples of one another, which, when dissolved in water, combine with a certain quantity of it, and which have an affinity for hydrochloric acid, this substance replacing water, or combining with the proteid instead of water. And further, that the combination of hydrochloric acid with them in the presence of pepsine—a ferment which probably renders the links binding together the primary proteid molecules more unstable,—this combination evolves sufficient energy to split up the compound molecule. The daughter molecules

combining with a greater percentage of acid than their parent, in a similar manner may be resolved into a younger generation. In fact, serum-albumen is usually the grandfather of peptone. Molecules of peptone may not always, however,—to carry the analogy further,—be twins: that is to say, two molecules of proto-albumose, combining with hydrochloric acid, may split into partly deutero-albumose, partly peptone. Perhaps the difference of the hemi- and the anti- compounds so commonly displayed in the text-books on physiology arises in this last way, though I must say that, when I determined to do so, I seemed to have little difficulty in simplifying the anti-compounds if I used a dialyser as an artificial stomach; not, however, if I proceeded in the ancient manner—namely, by means of a flask.

A discussion of the probable form and constitution of the primary molecule would just now be foreign to my purpose. The manner in which the molecule of HCl takes the place of water is also immaterial; the Cl probably takes the place of hydroxyl.

I said above, that washing coagulated albumen, which is combined with some hydrochloric acid, with water, removed in time most of, if not all, the acid. In like manner, washing albumoses or peptones to which acid is combined with alcohol, serves to remove the acid. The insolubility of these substances in alcohol being probably due to the removal of water and the formation of a closed ring, so, in the same way, it may remove the combined acid. A point of some note with regard to this is, that by washing acid albumen frequently with distilled water, one may remove all the acid from it, obtaining what I believe to be serum-albumen again.

Turning now more particularly to this acid albumen or to acid globulin, two of the most peculiar of the proteid series, I would direct attention to the following facts. If a solution containing acid albumen, to which has been added a drop of phenolphthalein, be carefully neutralised by the addition, drop by drop, of a decinormal solution of caustic soda, long before the fluid becomes neutral the first opalescence formed by the precipitation of the acid albumen appears. If soda be still added until the precipitation of the proteid occurs in flakes, it will be seen that it has as yet no red colour; it is not yet alkaline. Filter off the acid

albumen, the filtrate is acid. Add the acidity of the filtrate to that corresponding to the soda previously added, and a deficiency will be found proportionate to the amount of proteid removed. This deficiency is generally 5 or 6 per cent. The first opalescence occurs usually when the acid, still not neutralised, is about 9 or 10 per cent. of the acid albumen. The latter figure probably represents the first interference by the soda with the acid combined to the proteid; the first figure, the point at which some of the acid combined to all the acid albumen molecules is affected, leading to complete precipitation. Another point remains to be noticed, namely, that immediately after the complete precipitation of the acid albumen, if it be not filtered off, any further addition of the soda causes a solution of the previous precipitate, which has generally almost vanished before the solution becomes alkaline. Does the soda take the place of the acid, to some degree, before general alkalinity occurs?

These observations are justified by a group of experiments of a similar character to Nos. XII. and XIII.

Experiment XII.

One gramme of dried uncoagulated egg albumen with 50 ccms. of distilled water was placed in a dialyser, with 200 ccs. of a ·072 per cent. HCl solution outside. Next day there were 53 ccms. inside of an acidity of ·1116 per cent., ·0396 per cent. of which was free. 46 ccms. of this fluid was taken, containing ·491 gr. of acid albumen. A drop of phenolphthalein was added, and decinormal soda dropped into it, with the following result :—

HCl equivalent of soda added.	Result.
·03636 grm.	Precipitate beginning to fall.
·04458 ,,	Precipitate dense.
·048 ,,	Clearing up.
·05148 ,,	Clear, pink, just alkaline.

The total acid present then was ·05148 grm.: the proteid began to fall when ·01512 gr. HCl was still present. This amount of acid was required to keep it in solution. But beforehand, ·072 per cent. was combined to the albumen, or ·0156 grm. Therefore, whenever this amount of acid was interfered with, some of the proteid was precipitated. The whole of the proteid was precipitated when ·0069 grm. HCl still remained.

Experiment XIII.

·251 grm. and ·294 grm. of acid albumen were dissolved in 50 ccms. of a solution of hydrochloric acid, free acid being present. They

were then tested as before, one with deci-, the other with centi-normal soda solution. The total acidity of the first solution was lower than in the second, ·132 and ·209 per cent. respectively.

1. HCl equivalent in grms. of soda added.	2. HCl equivalent in grms. of soda added.	Result.
·054	·090576	Opalescence.
·05652	·094212	Opaque.
·05904	·097776	Flaky precipitate.
·06012	·09954	Beginning to clear.
·06282	·103572	Clearing.
·06624	·104686	Alkaline, almost clear.

Between the first precipitation and alkalinity in these two a very similar proportion exists. In No. 1, ·01224 grm. HCl, or 4·91 per cent.; in No. 2, ·01411 grm. HCl, or 4·79 per cent. The other figures closely correspond.

That the fluid is not neutral when acid albumen is precipitated in flakes, is proved by the following observation on No. 2 of the last experiment.

Experiment XIV.

After the fluid in No. 2 of Experiment XIII. had been rendered alkaline, it was acidified again, and the acid albumen again precipitated in flakes by the addition of alkali. It was now filtered, and the filter-paper washed several times with distilled water. The filtrate contained no proteid material, but was acid, containing ·00216 grm. HCl. The difference between the point at which the precipitate fell in flakes in Experiment XIII. No. 2, and the alkalinity point, was equal to ·00691 grm. of HCl. The removal of the acid albumen diminished this by ·00475 grm. This either represents acid attached to the proteid, or the equivalent amount of soda required to be taken up by the albuminoid before phenolphthalein is changed in colour by free alkali.

Acid globulin behaves in much the same manner, falling sooner, however, and persisting longer in an insoluble form, than the corresponding albumen.

The Effect of Dialysis on the Proteid Hydrochlorides.

On submitting albumen with which hydrochloric acid is combined to the action of dialysis into distilled water, the acid is removed, the albumen remains. If albumoses similarly combined are similarly treated, some of the albumose, more if it be the secondary albumose, dialyses, and with it the acid combined to it, and also the acid combined to the remainder left inside.

On the other hand, peptone and the acid combined with it dialyse out freely together. Again, if a solution of albumen with HCl combined, in which, however, there is no free acid present, be dialysed, free acid appears in the water outside and never more than a trace of acid in a state of combination, while if peptones be similarly treated, no free acid appears either outside or within. An interesting point occurs here, but before touching on it, I must contradict what I said above. I there made the statement that acid combined to proteid without the aid of pepsine is capable of further digestion on the addition of that ferment, while, conversely, acid which had saturated the proteid molecules under the influence of pepsine, was incapable of further digestion. This last statement is not correct, for I have been enabled to separate, in the presence of pepsine, the acid combined to albumoses after a regular digestive act by means of dialysis, and to split up these albumoses further into peptones by the action of the free acid thus obtained. The acid which is freed from some of the albumose molecules, probably those next the sides of the dialyser, is at once seized by adjacent molecules: these are able, owing to pepsine being present, to pick up the acid, they become over saturated, they split up, they form peptones. But as peptones require a greater percentage of acid than albumoses, the process can only go on for a short time,—the supply of acid is deficient.

Experiment XV.

25 ccms. of white of egg were digested for some hours with HCl and pepsine. The solution then contained the following constituents:—

Total solids,	1·81	per cent.	Total acidity, ·164 per cent.
Ash,	·09	,,	Free acidity, ·0
Organic solids,	1·72	,,	
Albumen,	·375	,,	
Acid albumen,	·0		
Albumose,	·6	,,	
Peptone,	·3184	,,	
	No free HCl.		

40 ccms. of such a solution were placed in two parchment tubes with 44 ccms. of distilled water added to each. ·015 grm. of pepsine was added to the first, the second was boiled. Round each, 200 ccms. of distilled water were poured. Each therefore contained ·724 grm. total solids, ·036 grm. ash, and ·680 grm. organic solids.

The two were kept at 38° Cent. for twenty-four hours. Next day, Outside—

1. 182 ccms. ; ·0175 per cent. HCl = ·03185 grm. HCl.
2. 183 ,, ; ·0173 ,, ,, = ·31659 ,,

1.	Total solids, .	·2971 grm.	
	Ash, . .	·0301 ,,	No Liebermann's reaction.
	Organic solids,	·267 ,,	
2.	Total solids, .	·2436 grm.	
	Ash, . .	·0328 ,,	Trace of free HCl as shown by
	Organic acids,	·2108 ,,	Liebermann's reaction.

Inside—

1. 79·75 ccms. ·03075 per cent. acidity. ·0245 grm. HCl.
2. 82 ,, ·0351 ,, ,, ·02878 ,,

No free HCl in either.

1.	Total solids, . . .	·3828 grm.	
	Ash, ...	·01196 ,,	
	Organic solids, . . .	·37084 ,,	
	Albumen, ...	·13706 ,,	·17 per cent.
2.	Total solids, . . .	·4428 grm.	
	Ash, ...	·0082 ,,	
	Organic solids, . . .	·4346 ,,	
	Albumen, ...	·1394 ,,	·17 per cent.

To sum up—

1 + Pepsine, .	inside,	·37084 grm.	organic solids.
	outside,	·267 ,,	,,
Total, .		·63784 ,,	,,
2 – Pepsine, .	inside,	·4346 grm.	organic solids.
	outside,	·2108 ,,	,,
Total, .		·6454 ,,	,,

As in both cases there was no free hydrochloric acid to start with, and as it had already done its work in digestion, it is clear that dialysis has the power of separating it from its proteid. In the example without pepsine, which had been previously boiled to preclude any error from the previous addition of this ferment, free HCl was found outside at the end of the experiment. In No. 1, on the other hand, the dialysable bodies were increased, and yet no free HCl could be discovered. Any free HCl that might have been liberated has, under the influence of the pepsine, attached itself to some nearly saturated proteid molecule, and split it into a simpler form. In this way the organic solids outside are increased considerably.

One or two similar experiments, performed on similar lines, corroborate this assertion, and need only be referred to here.

One was especially striking, as albumose and peptone were alone used in it, and a large proportion of the albumose was converted into peptone by a like process to the preceding.

Natural Digestion.

It will be seen from what I have said that during an act of gastric digestion all the albumen is not converted into albumose before that is turned into peptone. From the foregoing experiments it is plain that if a solution containing both serum and acid albumen be digested with HCl and pepsine, the acid albumen molecules are acted on first, and that, indeed, peptone may be formed long before any difference has occurred in the quantity of serum albumen present.

This accords with the fact that within a quarter of an hour after a meal of the higher proteids, all the simpler forms may be identified in the stomach contents; and that long before, comparatively speaking, any free HCl can be detected in those contents, the gastric digestion of proteids has, in part, progressed to its appointed end. The acid secreted by the stomach combines with the albumen and globulin; combining, splits them into simpler forms; and free acid still being secreted, these in turn are simplified, and in this process the free acid is used up. In time, however, the ratio of free HCl added to the stomach contents exceeds that which can combine, when naturally the presence of free acid can be demonstrated in the contents. Until this point is reached, generally an hour after food is taken,—the time varying, however, with the food,—all the free acid secreted by the gastric glands combines at once with proteids. The stages of gastric digestion, after a meal consisting chiefly of proteids, may be divided into—

1. Amylolytic stage, acidity slight, rising, no free HCl present, but some combined to proteids. Duration, about ten minutes. Peptones generally present.

2. Combined-hydrochloric acid stage, acidity considerable, rising, all forms of proteid present, no free HCl. Probably some lactic acid. Duration, until about the half hour.

3. Free hydrochloric acid stage, acidity still increasing, some

free HCl, mostly combined, lactic acid disappearing. From the half hour to three hours or so.

4. Last stage. Acidity falling, free acid rather in excess of the combined. From the third to the fifth hours.

Absorption probably goes on in varying degrees throughout; as albumoses dialyse, they possibly can be absorbed in their complete form. Indeed, as the rectum can absorb albumen, there is no reason why the stomach should not be able to absorb albumoses. Then, by some converse process, these bodies are changed into albumen again.

Pancreatic digestion, most energetic when there is considerable excess of fatty acids present, may be of much the same character: that I must leave for future investigation.