SCHEFFER
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PROPERTIES AND DIGESTIVE STRENGTH.

BY

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PEPSIN:

A NEW, PRACTICAL AND RELIABLE METHOD TO PREPARE IT; ITS PROPERTIES AND DIGESTIVE STRENGTH.

By E. Scheffer.

When publishing my paper upon Saccharated Pepsin (Amer. Journ. of Pharm. Jan., 1871) my intention was to continue the experiments, then only hinted at, and to publish the results. I have since made a large number of experiments, some of which I deem of sufficient importance to be made known, although my researches are not finished.

The various methods for the preparation of Pepsin, as given by different authors, seem to be intended mainly for the purpose of experiments, and are so complicated that the difference in the properties characterizing the products is readily accounted for. The student of physiology may not shun the trouble attending these processes, but the manufacturer could not possibly resort to them, even if he was so inclined and no doubt wished for a more simple and practical method.

The author of Leop. Gmelin's Hand-book of Chemistry, in the last volume of the work (issued in 1870) says, under the heading of Pepsin: "*The Pepsin of commerce is either mucus of the stomach, scraped off and dried, or a mixture of Pepsin, Peptons and Starch, containing a little lactic acid.*" In what way these commercial pepsins were prepared it is difficult to say, as most manufacturers have their own way and keep it a secret; but in Europe, as well as in this country, most of these preparations died almost as soon as they were brought into existence, as they did not come up to what they were represented to be.

In the summer of 1870, while working on and experimenting with *Liquid Pepsin* (Amer. Journal of Pharm. March, 1870,) and at the same time trying to improve it, I discovered some tests which I considered useful in the preparation of dry pepsin. Before this I had wished to prepare pepsin in the dry state, but was not inclined to follow the tedious and in some way uncertain processes usually given.

Following up the hint received by certain tests with a number of experiments, I succeeded at last to obtain a very satisfactory product.

The action of saturated solutions of some of the neutral salts of the alkalies on different protein substances induced me to try their effect on pepsin. For this purpose I prepared an extraction of the mucous membrane of fresh hogs stomachs with water, acidulated with muriatic acid, which after repeated filtrations formed an opalescent yellowish liquid. Equal volumes of this liquid and of a saturated solution of sulphate of soda, when well mixed together, formed a precipitate, which was collected on a filter, pressed and dried; a very small quantity of it, dissolved in water with the aid of a few drops of hydrochloric acid, dissolved coagulated albumen. Other saturated saline solutions were now experimented with, viz: of sulphate of magnesia and chloride of sodium, and also a solution of chloride of calcium of 1.27 spec. grav.

By these solutions precipitates were likewise found to form, possessing properties identical with that obtained by sulphate of soda, but I finally decided to employ chloride of sodium as the precipitant, as by a comparative test, which of the four different salts would produce the most precipitate, the proportion was: chloride of sodium 4, sulphate of magnesia 3½, sulphate of soda 2, chloride of calcium 1, so that chloride of sodium gave twice as much precipitate as sulphate af soda, and four times as much as chloride of calcium. But besides the larger yield, the sodium chloride has the preference for its antiseptic properties. A part of the precipitate, formed by sulphate of magnesia and allowed to remain in the liquid, had a putrid odor after the third day, while a moist precipitate, formed by chloride of sodium and set aside purposely for experiments, proved to be good after six months.

Preparation of Pepsin. On this basis I now began to prepare pepsin. Of the well cleaned fresh hog stomach the mucous membrane is dissected off, chopped finely and macerated in water, acidulated with muriatic acid, for several days, during which time the mass is frequently well stirred. The resulting liquid, after being strained, is, if not clear, set aside for at least twenty-four hours in order to allow the mucus to settle. To the clarified liquid the same bulk of a saturated solution of sodium chloride is added, and the whole thoroughly mixed. After several hours the pepsin, which by the addition of chloride of sodium has separated from its solution, is found floating on the surface, from whence it is removed with a spoon and put upon cotton cloth to drain ; finally it is submitted to strong pressure, to free it as much as possible from the salt solution.

The pepsin, when taken from the press and allowed to become air dry, is a very tough substance, and presents, according to thickness, a different appearance, resembling in thin sheets parchment paper, and in thick layers sole leather ; its color varies from a dim straw yellow to a brownish yellow. Besides a little mucus it contains a small quantity of phosphate of lime and chloride of sodium, which, however, do not interfere with its digestive properties, as they are found also in normal gastric juice.

SACCHARATED PEPSIN. To work it into *Saccharated Pepsin* (Am. Journ. of Pharm. January 1871) the damp pepsin, as it is taken from the press, is triturated with a weighed quantity of sugar of milk to a fine powder, which, when having become air dry, is weighed again, the quantity of milk-sugar subtracted and so the amount of pepsin found. The strength of this dry Pepsin is now ascertained by finding how much coagulated albumen it will dissolve at a temperature of 100° F. in five or six hours, and after this sufficient milk sugar is added to result in a preparation of which ten grains will dissolve one hundred and twenty grains of coagulated albumen, and this preparation I have called *Saccharated Pepsin.*

PURIFICATION OF PEPSIN. Anxious to get the pepsin in its purest state, if possible, chemically pure, I tried different methods, but have not succeeded as yet. In order to get a purer article I re-dissolve the pepsin, as obtained after expression, in acidulated water, filter the solution through paper, and precipitate again with a solution of sodium chloride ; the precipitate, after draining and pressing, is now free of phosphate of lime and mucus, but contains yet salt. In the freshly precipitated state the pepsin is very readily soluble in water and cannot therefore be freed from adhering salt by washing.

By allowing the pressed sheet of Pepsin to get perfectly air dry—whereby it becomes coated with a white film and small crystals of chloride of sodium—and by immersing it then in pure water for a short time, the greater part of sodium chloride can be extracted, but it has to be done very rapidly, as the pepsin swells up considerably and looses its tenacity. By operating in this manner I obtained a pepsin which dissolves in acidulated water to quite a clear colorless liquid, but as it still contains traces of salt, I preferred to call it *Purified Pepsin.*

I obtained a pepsin quite free of chloride of sodium—which by combustion did not leave any ashes—by swelling purified pepsin in water to a thick mucilaginous liquid and mixing it with alcohol of 95 per cent. A gelatinous almost transparent precipitate is formed, which is put on a cloth, washed with diluted alcohol, then pressed and dried. This preparation did not leave any ashes by combustion, but I was greatly disappointed in my expectation, when I found that the digestive strength of this pure pepsin was not as great as that of the purified pepsin, which still contains sodium chloride. No doubt the use of alcohol had impaired the digestive power of the pepsin to some extent.

PROPERTIES OF PEPSIN. The pepsin is, as already mentioned, very soluble in water, when recently precipitated, but when once air dry dissolves but slowly and only in very small quantities in water.

The dry purified pepsin, when put into water, swells up considerably, becomes perfectly white and, when vigorously shaken, disintegrates to small floccules, which swim in the liquid and remain suspended for a long time, while a very small quantity will dissolve.

The watery solution has an almost neutral reaction, is coagulated by boiling, and gives with alcohol a transparent, gelatinous precipitate.

With sulphate of copper it remains clear at first, but after several hours becomes turbid.

Bi-chloride of mercury gives immediately a white precipitate.

With tannin a very copious white precipitate is obtained.

Nitrate of lead forms a white precipitate.

The precipitate, formed by chloride of sodium, is very characteristic and at the same time very interesting. When a saturated solution of chloride of sodium is added to a clear solution of pepsin, not too concentrated, at first a jelly-like transparent coagulation is formed, which disappears upon stirring, and the liquid acquires a slightly opalescent appearance; after a short time it becomes more turbid and small flakes are noticed floating in it, which soon will form into small transparent globules and as such rise to the surface. When the quantity of pepsin in a liquid is very small, the opalescence and turbidity is hardly noticed, but after some time the small globules will appear on the surface.

The watery solution of pepsin decomposes readily; after a few days small flakes separate from the clear solution, which increase in number by longer standing, and on the fourth day already it emits a foul disagreeable odor.

The watery solution of pepsin shows very little action on coagulated albumen; a certain quantity of albumen, which by a watery solution was hardly acted upon in twenty-four hours, was readily dissolved, after addition of a few drops of hydrochloric acid. A watery extraction of the mucous membrane was also experimented with, with the same result; before the addition of hydrochloric acid it did not dissolve albumen; after acidulating it the albumen dissolved easily.

PROPERTIES OF ACIDULATED PEPSIN. An acidulated solution of pepsin was made of such strength, that one fluid-ounce contained one grain of purified pepsin and two drops of hydrochloric acid, and experimented with.

By boiling, the clear liquid becomes turbid and, upon cooling, deposits flakes.

By addition of alcohol it remains clear at first, but upon standing, flakes of pepsin separate from it.

Strong hydrochloric acid produces slight turbidity, which disappears by addition of more acid or by dilution with water.

Chloride of sodium gives the characteristic precipitate.

Bi-chloride of mercury produces opalescence.

Tannin forms a heavy precipitate, soluble in hydrochloric acid.

Gallic acid shows no action.

Carbonate and bi-carbonate of soda produce a precipitate soluble in excess.

MODIFIED PEPSIN.—A solution of carbonate of soda carefully added to a solution of pepsin produces a precipitate which, upon being separated from the liquid, will prove to be pepsin; but a little more of carbonate of soda will redissolve it again, and the liquid no longer contains pepsin; that is, the pepsin is destroyed or modified.

This circumstance caused me to say in my essay (Amer. Journ. of Ph. 1871, page 6,) "*dry pepsin, precipitated with alcohol from its solution, did not act at all on albumen,*" which remark I herewith revoke as erroneous. The fact was that, intending to make pure pepsin and not getting a precipitate by alcohol in the sour solution, I added carbonate of soda to neutralize the acid, and then obtained by alcohol a precipitate which I believed to be pure pepsin; at that time I had not studied the change which carbonate of soda produces in pepsin.

When I say above the pepsin is destroyed I mean its action on fresh coagulated albumen. A pepsin solution, made entirely neutral, or rather a little alkaline by addition of carbonate of soda, which afterwards is acidulated again with hydrochloric acid, has lost its power to dissolve fresh coagulated albumen.

The alkaline solution assumes a foul odor after a short time; it does not act on fresh coagulated albumen, except when putrification sets in, and then the more putrid the solution becomes, the more it seems to act on albumen; at the same time the most natural odor of healthy human fæces will show itself.

But, on the other hand, the alkaline solution, by itself as well as when acidulated, dissolves *partly digested* albumen.

Coagulated albumen, put into pepsin solution until half gone, then taken out on a cloth and washed and put into an alkaline pepsin solution, will dissolve; it will likewise dissolve in an alkaline solution which has been again acidulated by the addition of hydrochloric acid. But these solutions have a different appearance from a solution by pepsin; they are not as clear and thin a liquid as the latter.

An alkaline (modified) pepsin solution does not get precipitated by chloride of sodium, but by addition of hydrochloric acid immediately a copious gelatinous precipitate will be formed.

Digestive power of Pepsin.—In my former experiments the strength of pepsin was ascertained by allowing its solution at a certain temperature to act upon a convenient quantity of coagulated albumen for a given time, and determining the quantity dissolved by weighing that undissolved; the albumen by this method was only partially dissolved. In my recent experiments I determined the strength by ascertaining the amount of albumen that would be fully dissolved in a certain time and at a given temperature. I had found that the solvent power of pepsin is not inverse proportional to the time; for if a pepsin dissolves X albumen in S time, $2a$ pepsin will not dissolve X albumen in $\frac{S}{2}$ time, as might be supposed, but require longer time. The last portion of coagulated albumen to be dissolved in an experiment requires much longer time in proportion, even when pepsin is in excess.

Having used heretofore, in my experiments with pepsin, 10 drops of hydrochloric acid to the fluidounce of water, I wished to determine whether or not a smaller quantity of acid would answer the same purpose. It was of importance to ascertain if by the preparation of *liquid pepsin* a smaller quantity of acid would produce the same results, as some complaints were made of the acidity of that preparation as first prepared.

Of four experiments, in which a certain quantity of pepsin was dissolved in 1 ounce of water with respectively 4, 6, 8 and 10 drops of hydrochloric acid of 1·17 spec. grav., the same amount of coagulated albumen was dissolved in the shortest time where 6 drops, then where 8 drops and, thirdly, where 10 drops of acid were employed, while the experiment containing 4 drops of acid had, after 6 hours, a considerable quantity of albumen not dissolved. I, therefore, made all my subsequent experiments with a solution containing 6 drops of hydrochloric acid to the fluidounce of water, at a temperature of 100 to 105° F., and each vial was shaken about every 10 minutes.

One grain of purified pepsin in 4 oz. of acidulated water was found to dissolve 400 grs. of coagulated albumen in 18 hours at 75° F.

One grain of purified pepsin in 4 oz. of acidulated water dissolves 500 grs. coagulated albumen at a temperature of 105° F., in 6 hours.

Ten (10) grains of saccharated pepsin dissolve 120 grs. of coagulated albumen in 4 to 6 hours at 100° F.

Although I did not succeed to prepare a pepsin like Wasman's, of which 1 part was capable of dissolving 60,000 parts of coagulated albumen I found that the digestive power of pepsin was almost inexhaustible.

With one-half grain of purified pepsin in 2 oz. of acidulated water I dissolved 250 grains of coag. alb.; to the solution was added another oz. of acidulated water and 250 grs. of albumen; when it was again dissolved I added in these fractional proportions of acidulated water and albumen, until finally the one-half grain had dissolved 1500 grains of coagulated albumen. That it would have dissolved still more I proved in an experiment, mentioned hereafter.

PEPTON SOLUTION.—When the albumen, which by the digestive process is converted into albuminose or pepton, is perfectly dissolved, the resulting pepton solution is a very limpid, thin, slightly yellowish-colored liquid, which, when filtered, has an opalescent appearance.

By addition of alcohol it remains at first clear, but forms, after 24 hours, a gelatinous precipitate.

Pepton precipitate.—An equal volume of saturated salt solution added to the pepton solution produces a copious, perfectly white precipitate, which, upon being collected on a filter, drained, pressed and dried, yields a hard white substance containing pepsin, peptons, chloride of sodium and a little acid. Put into water it becomes translucent, like horn, and dissolves after some time.

Its solution has an acid reaction; is not coagulated by heat; hydrochloric acid produces a heavy precipitate which, by dilution with water or by addition of more acid, will redissolve; with alcohol it becomes opalescent and forms after some time a precipitate.

Bichloride of mercury gives a heavy white precipitate.

Coagulated albumen put into the watery solution is hardly acted upon, but when acidulated with hydrochloric acid it is dissolved.

Digestive power of the Pepton precipitate.—The digestive power of the precipitate, obtained by addition of sodium chloride to the pepton solution is remarkable. In many cases a solution of one grain of the precipitate in one oz. of acidulated water dissolved 100 grains of coagulated albumen.

With 20 grains of saccharated pepsin in 2 oz. of acidulated water I dissolved 240 grs. of coagulated albumen; the precipitate obtained from this solution by chloride of sodium weighed, when dry, 12 grains, of which 1 grain dissolved 100 grs. of coagulated albumen; from this last solution again, by chloride of sodium, 10 grains of precipitate were obtained, of which 1 grain dissolved between 20 and 30 grs. of coagulated albumen. In this way the 20 grs. of saccharated pepsin, for which I only claim the power to dissolve 240 grs. of albumen in 6 hours, dissolved at the rate of between 4000 and 5000 grains.

The solution of 1500 grs. of albumen, obtained by fractional addition of albumen and acidulated water to an acidulated solution of half a grain of purified pepsin, mentioned above, furnished with chloride of sodium a precipitate, which also had considerable digestive power.

Relation of Chloride of Sodium to the digestive power of Pepsin.—By its preparation the commercial, saccharated pepsin contains always a small quantity of chloride of sodium; in my experiment, to obtain a pure pepsin free of sodium chloride, I succeeded by using alcohol, but the resulting product had less digestive power than purified pepsin, which still contains salt. It was, therefore, interesting to determine if chloride of sodium would aid the action of pepsin on albumen and accelerate its solution.

A very small quantity of salt, a quantity that does not exceed much that of the purified pepsin, does not interfere with, on the contrary benefits the pepsin in its action; but a larger quantity, although very small in itself, retards the solvent power.

While half a grain of pure pepsin in 2 oz. of acidulated water dissolved 200 grains of coag. alb. perfectly, a great deal of albumen was left undissolved in the same time when 5 grs. of salt were added to it, while by 10 grains of salt a portion of the albumen was not dissolved after three days.

STABILITY OF PEPSIN.—As watery solutions of pepsin decompose very soon, particularly in warm weather, it was of interest to determine the stability of acidulated solutions; accordingly solutions containing one grain of purified pepsin to the fluidounce of water, and respectively 2, 4, 6, 8 and 10 drops of hydrochloric acid were set aside, a portion of each in well-corked vials and another portion in vials only tied up with paper. The solutions containing 2 drops of acid became mouldy after the first and second week, while in the vials, with 4 drops of acid, I noticed mould after five weeks. The other solutions kept entirely clear, and when examined, after 6 months, they did not have any bad odor, but had lost their digestive power almost entirely; albumen, put into several of the solutions, was hardly acted upon, and chloride of sodium did not produce the characteristic precipitate.

To 20 grs. of purified pepsin, swelled in 2 ounces of water, were added 10 drops of hydrochloric acid, which dissolved the pepsin fully and formed a liquid of a slight yellowish color, and the consistence of the officinal mucilage of gum arabic. Put aside in a beaker-glass, tied up with blotting paper, it evaporated slowly, and was, after 6 weeks, dried out to a transparent gum which felt sticky to the touch. Examined after several months, it dissolved readily in water, forming a clear solution of sour reaction and taste, which had no bad odor, but acidulated and diluted to the strength usually employed in my experiments, did not act on coagulated albumen, and chloride of sodium gave no precipitate. The pepsin was therefore totally destroyed or at least made inactive.

Anxious to learn whether *liquid pepsin*, which had been put aside eight months before for experiments' sake, had retained its digestive properties, I examined this and found that, although slower in its action, it still dissolved albumen, and was also precipitated by chloride of sodium.

It seems, therefore, that the glycerin in the preparation of liquid pepsin prevented the pepsin from decomposition.

In the spring I had set aside moist precipitate (by chloride of sodium) of pepsin of the consistence ready for the press; when examined after six months it had a sweet odor, was pressed, dried, and its digestive power ascertained, whereby it proved to have the same digestive strength as when fresh prepared.

Several times the (chloride of sodium) precipitate, while draining on the cloth, was entirely frozen through, but proved, after thawing not inferior in quality.

The purified as well as the saccharated pepsin, examined twelve months after preparation, proved to be entirely as good as when recently prepared; they had lost nothing of their strength, and dissolved albumen in the same time and in the same quantities as when quite fresh. The only difference is, that with age the dry pepsin dissolves somewhat slower in acidulated water.

ACTION OF PEPSIN ON MILK.—As the opinion is still prevalent, even amongst physicians, that only calf rennet has the property of separating the casein from the milk, or, in other words, to coagulate milk, it was interesting to me to try the action of pepsin on milk.

Five grains of saccharated pepsin, swelled in a little water and then stirred into one pint of milk, coagulated the milk in 30 minutes.

Of a solution of two grains of purified pepsin, two drops of hydro chloric acid and one fluid ounce of water, it took five drops to coagulate four ounces of milk in about 20 to 30 minutes; while 10 drops of dilute muriatic acid (20 drops to one oz. of water) did not curdle four oz. of milk in four hours.

Averaging 400 drops in a fluid-ounce of the pepsin solution, it took one-fortieth ($\frac{1}{40}$) part of one grain to coagulate four ounces of milk or one grain to five quarts; according to this test, one part of pepsin will coagulate about 80,000 (eighty thousand) parts of milk.

The success of these experiments depends a great deal on the temperature; the best way is to add the pepsin to the milk when cold, and then heat it slowly; when kept cold it takes much longer time to coagulate the milk. Also when the milk is heated first, say to

100° F., before the pepsin is added, it takes three to four times as much pepsin to effect coagulation.

ALCOHOL INCOMPATIBLE WITH PEPSIN.—In my former articles written about pepsin, I have mentioned the incompatibility of pepsin and alcohol, and have spoken of the impropriety of dispensing pepsin in the form of wine or elixir. Having now a purer pepsin at my disposal than before, I repeated the experiments with entirely the same result.

Seven vials of solution of pepsin, each containing the same amount of pepsin and hydrochloric acid, were made with that difference, that, while vial No. 1 contained only one fluid-ounce of water, No. 2 contained one-half drachm of alcohol and seven and a half drachms of water; No. 3, one drachm of alcohol and seven drachms of water; and so each following vial one-half drachm of alcohol more than the preceding one, so that in vial No. 7 there were five drachms of water and three drachms of alcohol. The same amount of coagulated albumen was given into each vial, which were exposed then to a temperature of 100° F. After six hours in vial No. 1 all the albumen was dissolved; in No. 2 some albumen was left undissolved, No. 3 contained more, and in No. 4 over half of the albumen was not dissolved, while in five, six and seven the albumen was a little changed in appearance, but the bulk not diminished. The contents of those vials in which the albumen was not much acted upon, emitted that peculiar sour odor which characterizes discharges of an overloaded stomach (with beer or wine) by vomiting.

A solution of half a grain of purified pepsin in half a fluid-ounce of water, with three drops of hydrochloric acid, was mixed with one fluid-ounce of sherry wine, after 24 hours filtered, and then, with the addition of 150 grs. of coag. albumen, exposed to a temperature of 105° F. After six hours—during which time the half grain of purified pepsin in acidulated watery solution would have dissolved 250 grs. of coag. albumen—of the 150 grs. at least two-thirds yet remained. I added now six drops more of hydrochloric acid to bring the liquid to my standard acidity, but even at the end of 24 hours a large quantity of the albumen was undissolved.

Having never made pepsin by any other method, I am not able nor justified to judge between the different products; but that my process excels by simplicity, nobody will question. That a complicated process, by which strong bodies, as mercury, lead and sulphuretted hydrogen, are alternately used, to prepare a substance, should or might impair the quality of the product, is very probable. That nevertheless pepsin, prepared by such a method, has the digestive power, speaks for the almost inexhaustible strength of it.

Another point of importance in my preparation I would call attention to, is that no artificial heat at all is used, neither by extracting the stomachs nor by drying the pepsin, and in my whole process no evaporation is necessary. To evaporate the solution of a substance, for which a few degrees difference in heat decide between life and death, is a very delicate operation, which is easily carried out for experimental purposes, but on a larger scale is almost impossible.

My pepsin differs from the pepsin described in Gmelin's Handbook, principally by the latter being easily soluble in water, while mine, although very soluble in the moist state, looses its solubility almost entirely by exsiccation.

That pepsin precipitate, which, combined with pepton, I obtained from the pepton solution, is more identical to the pepsin described in Gmelin's Handbook (Volume 8, Zoochemie), as it is easily soluble after having become dry, is completely precipitated by alcohol, shows a more acid reaction and its clear solution becomes more turbid by addition of hydrochloric acid than the pure pepsin.

To bring the pepsin into a finely divided state, I preferred the use of milk sugar to that of starch, the substance generally used for this purpose, particularly by the French manufacturers; reasoning that sugar with its antiseptic properties will contribute to the stability of it, while starch, particularly in the damp state, is very apt to get mouldy, and will then, as a necessary consequence, cause the decomposition of the pepsin.

When first making the commercial pepsin, which I called *saccharated pepsin*, I aimed to make it of such strength that one grain of the saccharated should correspond in its digestive power to one teaspoonful of the *liquid pepsin* (Amer. Journal of Phar., January, 1871); that it can be made of much greater power I have plainly shown by the before mentioned results.

As for the precise strength that will be best suited for the human stomach, that will have to be determined by physiologists. According to Schroeder, the normal gastric juice of man dissolves 24 per cent. of coagulated albumen; five grains of saccharated pepsin, which in acidulated solution dissolve 60 grs. of coagulated albumen in four to six hours would correspond to half an ounce of human gastric juice. No doubt the beneficial effect of pepsin has its limits. Several grains of the purified pepsin, of which one grain dissolves 500 grs. of albumen in six hours, might do more harm in the human stomach than good, and might even do positive injury.

But, in this essay, I have given only facts based on chemical experiments; to make use of these facts for therapeutical and physiological purposes, I leave to physicians.

Louisville, *Ky*., *January*, 1872.

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