THE RETARDING EFFECT OF CERTAIN SUBSTANCES ON PEPSIN DIGESTION.*

BY C. F. RAMSAY.

The author finds that comparatively small amounts of quite a number of salts in combination with pepsin interfere or negative the test for pepsin, but that in the preparations of the National Formulary the added constituents of compound preparations of pepsin do not inhibit the activity of the pepsin, at least in so far as the test is concerned.

During the course of some work which required the testing of a number of tablets and elixirs containing pepsin it was found that some of these samples did not have the pepsin activity which they should have possessed. Some of the tablets contained a sufficient quantity of calcium carbonate to lessen the acidity of the acid medium, thus making the tablets appear to contain less pepsin than was really present. In other samples there were present substances which by their mere presence retarded the pepsin digestion.

In considering the retarding effect of certain substances on pepsin digestion we must bear in mind the extremely small amount of pepsin that is needed to bring about the transformation of a large amount of albumen. For instance, if a sample of pepsin is 1 in 3000 U. S. P., then one gramme would digest 3000 grammes of egg albumen. Ten grammes of albumen, the amount used in the U. S. P. test, would require only 0.0033 gramme of pepsin to digest it. If one fluidrachm of a pepsin solution digests 1200 grains of albumen, then ten grammes of albumen would require only 0.47 Cc. of this solution. The small amount of pepsin required to digest a large amount of albumen probably accounts for its sensitiveness toward other substances.

The writer finds that pepsin is quite sensitive toward iron and bismuth. In the N. F. Elixir of Pepsin and Iron there is no retarding effect from the amount of citro chloride of iron specified, but if twice as much iron is used the pepsin tests only 1 in 2250, or 75 percent of what it should, and if four times the amount is used the pepsin tests only 1 in 1800, or 60 percent of what it should. In the Elixir of Pepsin and Bismuth N. F. there is no retardation, but if twice as much glycerite of bismuth is used the pepsin tests only 1 in 2250, or 75 percent of what it should, and using three times the amount of bismuth the pepsin tests only 1 in 1800, or 60 percent. In determining the retarding effect of substances on pepsin digestion, the writer placed the amount of the pepsin 1 to 3000 required for ten grammes of egg albumen into each of ten bottles containing the albumen. Three of these bottles were reserved as the standards, and to the other seven were added different amounts of the substances being tried out for their retarding effect. It was first determined how much of the substance had to be added to show an appreciable retardation. Then larger amounts were used. For instance, adding 0.01 gramme of potassium and sodium tartrate to 10 grammes of egg albumen, the strength of the pepsin was not affected, but adding 0.02 gramme made it test 1 in 2750, and with 0.03 gramme it tested only 1 in 2500. The retarding effect of the following substances was carried out in the same manner:

* Read before Scientific Section, A. Ph. A., San Francisco meeting.

Sodium Phosphate.

0.01 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.02 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.03 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.04 gramme caused the pepsin to test 1 in 2250, or 75.0 percent

Magnesium Sulphate.

0.0025 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.01 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.02 gramme caused the pepsin to test 1 in 2250, or 75.0 percent
0.03 gramme caused the pepsin to test 1 in 2000, or 66.6 percent

Ammonium Chloride.

0.02 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.03 gramme caused the pepsin to test 1 in 2250, or 75.0 percent
0.04 gramme caused the pepsin to test 1 in 2250, or 75.0 percent

Sodium Chloride.

0.03 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.05 gramme caused the pepsin to test 1 in 2250, or 75.0 percent
0.07 gramme caused the pepsin to test 1 in 1750, or 58.0 percent

Calcium Chloride.

0.01 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.02 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.03 gramme caused the pepsin to test 1 in 2250, or 75.0 percent
0.04 gramme caused the pepsin to test 1 in 2000, or 66.6 percent

Potassium Chloride.

0.01 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.02 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.03 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.04 gramme caused the pepsin to test 1 in 2250, or 75.0 percent

Magnesium Chloride.

0.03 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.04 gramme caused the pepsin to test 1 in 2750, or 91.7 percent

Manganese Chloride.

0.02 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.03 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.04 gramme caused the pepsin to test 1 in 2500, or 83.4 percent

Aluminum Chloride.

0.02 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.03 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.04 gramme caused the pepsin to test 1 in 2500, or 83.4 percent

Saccharin.

0.01 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.02 gramme caused the pepsin to test 1 in 2250, or 75.0 percent

Cane-sugar.

No retarding effect using as much as 0.05 gramme.

Tannic Acid.

0.05 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.1 gramme caused the pepsin to test 1 in 2500, or 83.4 percent
0.2 gramme caused the pepsin to test 1 in 2000, or 66.6 percent

Chloroform.

0.1 Cc. makes it almost inert.

Nicotine.

0.02 Cc. pure nicotine retards the pepsin slightly. Above this the retardation is very marked.

Pancreatin.

0.03 gramme caused the pepsin to test 1 in 2750, or 91.7 percent
0.04 gramme caused the pepsin to test 1 in 2750, or 91.7 percent

Papain.

0.04 gramme caused no effect.

Glycerin.

0.8 gramme has no effect.

Alcohol.

1.5 Cc. had no effect.

Alkaloids.

With strychnine alkaloid, citrate, and sulphate there was no retardation, using as much as 0.04 gramme.

Caffeine showed no effect.

Morphine had no influence unless 0.01 gramme was used.

0.02 gramme quinine hydrochloride caused the standard to test 1 in 2750.

0.005 gramme of quinine sulphate caused the standard to test 1 in 2750, and with 0.02 gramme it tested 1 in 2500.

Spices.

Powdered ginger, cloves, nutmeg, and cinnamon had no effect.

From the above results we find that the N. F. Elixirs of Iron and Pepsin and Bismuth and Pepsin do not contain enough iron or bismuth to cause a retarding effect in the testing of these elixirs, but if the amounts of iron or bismuth were doubled the test would show less pepsin than is really present, because of the action of the metallic salts on the pepsin in the test. A combination of pepsin with other salts is not very common, but it may occur in prescribing to satisfy special conditions. It is to be noted that pepsin digestion is interfered with whenever any of the common medicinal salts are present to the extent of about three times the amount of pepsin. The sulphates have a greater retarding action than the other salts tried, but magnesium sulphate appears to be even more poisonous to pepsin

than the others. In addition to the above, it must be remembered that any salt which is present in sufficient quantity to lessen the acidity of the acid medium will make the pepsin appear less active, and if sufficient to neutralize the acid the pepsin will be rendered inactive.

As regards organic bodies, we find that cane-sugar has no effect, while saccharin has a decided influence.

Glycerin, alcohol, common alkaloids, spices, pancreatin, or papain has no influence on the test in proportions that are likely to be found in medicinal preparations.

Tannic acid and chloroform are quite injurious to pepsin, even in small proportions.

Very small amounts of nicotine show a decided injurious action, in contrast to the other alkaloids tried.

In conclusion, it must be remembered that in the testing of pharmaceutical preparations containing pepsin for pepsin activity or content the presence of inorganic salts is not a matter of indifference in the test, but must be taken into consideration. If these salts are present in sufficient quantity to reduce the action of the pepsin, the test will show less pepsin than is really present. In such cases the solution should be diluted to the point where the salt will be below the amount which influences the pepsin digestion. There is no evidence that the pepsin is killed in the original preparation by the salts, but the test is rendered inaccurate by the presence of these if in large enough proportions.

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VALUATION OF RHAMNUS BARKS.

Tunmann (*Apoth. Zeit.*) gives the following simple method for estimating the anthraquinone derivatives in barks of the various species of rhamnus. The dry, finely powdered bark (1.6 grammes) is boiled with 100 grammes of 4 percent sodium hydroxide solution in the case of rhamnus purshiana (or with a 3 percent alkaline solution in the case of rhamnus catharticus) and the mixture shaken for 10 minutes. After allowing the drug to settle the liquid is decanted upon a double filter, the residue shaken again for 10 minutes with 60 grammes of the caustic soda solution, the liquid filtered also and the filter and residue washed with 10 Cc. (mils) of caustic soda solution and water. The combined alkaline liquids are acidulated with hydrochloric acid and shaken for 30 minutes with 160 grammes of chloroform. The mixture is allowed to stand for two hours; 120 grammes of the chloroform solution are filtered off and shaken out with 120 grammes of caustic soda solution. After allowing to separate, 100 grammes of the alkaline solution are filtered, acidulated with hydrochloric acid and allowed to stand over night. The precipitate is then collected on a tared filter, washed with water acidulated with hydrochloric acid and dried at 60° C. to constant weight. The weight multiplied by 100 gives the percentage of anthraquinone derivatives in the bark. By this process rhamnus frangula assayed 3.8 percent; rhamnus carniolica, 4.1 percent; rhamnus purshiana, 1.8 percent, and rhamnus catharticus, 2.01 percent.—Through *Druggists' Circular.*