ARTIFICIAL DIGESTION EXPERIMENTS.[1]

By Edward Gudeman.

Received June 23, 1905.

Studies on the influence of colors, preservatives or condiments in digestion experiments are generally made in one of two ways, by direct feeding to man or animal or by artificial digestion tests.

The main objection to the feeding experiment is, that generally the quantities introduced into the system are greatly in excess of what is found normally in food products and often ill effects, if noticed, are liable to be due to this excess and in long-continued exper ments due to a cumulative action of the excess. When the quantities are reduced to approximately what is found in the average food products, such effects are not observed, especially if the color, preservative or condiment contain no directly physiologically active principles and are non-cumulative. The abnormal effects often observed during the administration of colors and preservatives, in small amounts, extending over a long period, differ in no way from the effects resulting from many of the commonly called harmless condiments such as spices, sugar, salt, vinegar, alcohol, vegetables colors, etc., when given under like conditions as to quantities and time, and such abnormal effects produced in the living organism are no criterion by which to judge the wholesomeness or harmfulness of the substances themselves.

The strongest objection urged against the artificial digestion experiments is, that such tests do not duplicate the complex action of the organized system and therefore are not at all conclusive as to what the effect of a color, preservative or condiment will be when consumed in a food product or with a food product.

Such experiments show in a directly numerical comparative way what the action is on digestive ferments and it is allowable to conclude that a similar relative effect will be produced in the organized system. A large number of artificial digestion experiments have been made by me to determine the relative action of substances not natural constituents of food products, as to retarding or increasing the digestive power of the ferments pepsin and pancreatin.

Experiments were made with uncoagulated egg albumen, with potato starch and a high proteid maize gluten flour. The egg albumen was specially prepared by taking the whites of a large

[1] Read at the Buffalo Meeting of the American Chemical Society.

number of fresh eggs straining through fine cheese-cloth, spreading on glass plates and drying in a current of air at temperature below 40° C. The starch was prepared from picked sound potatoes, washed until free from soluble material and air-dried at about 30° C. The maize gluten flour was prepared from commercial gluten meal treating with malt extract, washing to free it from soluble substances and drying at about 40° C. During the process of preparing this meal, it was heated to 100° C. to destroy any possible action of the malt diastase.

Great difficulties were encountered with the potato starch and maize gluten flour experiments, it not always being possible to separate the undigested portions. The action of the ferments was slow and not uniform, varying from day to day, under conditions as nearly identical as it was possible to maintain in a series of experiments extending over many months. The results obtained agreed fairly well with those on the egg albumen, but as only a limited number of these tests were successfully completed, the results are not enumerated and have not been taken into consideration in the conclusions drawn.

The character of the egg albumen, whether neutral, alkaline or acid, materially affects the results. Alkaline solutions, caustic or carbonated, cannot be used, all results being abnormal, depending on the amount of alkali present. Neutral solutions can only be used if the substances tested are neutral themselves or acid. The acid egg albumen solutions give best results and are the most rational ones, as these are similar in character to the stomach juices.

The standard solution used for these tests contained 2 per cent. dry uncoagulated egg albumen and 0.1 per cent. hydrochloric acid and was always freshly and daily prepared for each series of experiments, from a stock of dry egg albumen.

Sulphuric and phosphoric acids were found to act as well as hydrochloric acid, but preference was given to the latter, being the characteristic acid of stomach juices. In the experiments with colors, preference was given to acetic acid, as its solvent action on many colors was found to be less than that of the mineral acids.

The egg albumen was dissolved in water, to this was added sufficient 5 per cent. acid to give the required acidity, then the substance under examination, either dry or suspended or dissolved in a minimum amount of water and finally the required amount of the ferment. The mixtures were thoroughly stirred

and placed in a water-bath and kept at 35° C. for exactly one hour. The temperature was then quickly raised and kept at boiling for exactly one-half hour. The hot solution was poured on a weighed filter, well washed with boiling water, the residue dried at 100° C. and weighed. The difference in weight of egg albumen used and residue found, was taken as the amount digested. The pepsin or pancreatin were suspended in water and measured out, generally using one part of ferment to two thousand parts of the egg albumen. In no case was sufficient ferment used to completely digest all of the egg albumen. No relative differences were found between the action of the pepsin and pancreatin or the mixture of pepsin and pancreatin. Pancreatin reacts more slowly than pepsin or a mixture of the two. The proportional interference with digestion was the same for the two ferments, and the data given for pepsin hold good for pancreatin. The actual amount of egg albumen digested varies with the ferments and an increase of ferment decreases the interference in every case. Two or more blank checks were run with every daily set of tests and the amount digested of the blanks taken as 1000 and all other data calculated to this basis. Results obtained are given, the ratio at head of tables giving the proportion of egg albumen digested (1000) to substances tested (1, 2, 4, 10 and 25).

WITH PEPSIN IN ACID SOLUTION.

	1000:1.	1000:2.	1000:4.	1000:10.	1000:25.
Check blanks	1000	1000	1000	1000	1000
Salicylic acid	1000	970	920	800	700
Benzoic acid	1000	1000	970	900	750
Boric acid	1000	1000	970	940	850
Sulphurous acid	1000	1000	960	880	830
Acid sulphite of soda	1000	1000	960	900	850
Saccharin	1000	1000	1000	1000	1000
Sugar	1000	1000	1000	1000	1000
Vinegar (cider)	1000	1000	990	980	970
Alcohol, ethyl	1000	1000	980	950	930
Alcohol, methyl	1000	1000	1000	980	940
Salt (NaCl)	1000	1000	1000	1000	950
Formaldehyde	950	900	.....	.....	.....
Smoke[1]	900	.....	.....	.....	.....
Condensed smoke	900	750	500	.....	.....
Creosote	800	700	400	.....	.....

[1] The amount of smoke was not determined, being passed into the solution from destructive distillation of hickory chips. A commercial product "Condensed Smoke" was found to contain a large percentage of creosote, oils and other products of destructive distillation. No doubt the strong interference with the action of the ferment is mainly due to the creosote.

The results obtained in acid solution with the sodium salts of salicylic, benzoic, boric and sulphurous acids were identical with those obtained with the free acids, using sufficient of the salts to give the calculated amount of free acids. Used weight for weight the interference of the salts was slightly less.

WITH PEPSIN IN NEUTRAL SOLUTION.

	1000:1.	1000:4.
Check blanks	1000	1000
Salicylic acid	1130	1050
Benzoic acid	1150	1080
Boric acid	1150	1080
Sulphurous acid	1130	1070
Saccharin	1000	1000
Sugar	1000	1000
Condensed smoke	850	700
Salt	1000	1000
Vinegar	1150	1100
Hydrochloric acid	1150	1100
Sulphuric acid	1150	1100
Phosphoric acid	1150	1100
Nitric acid	1100	950
Sodium salicylate	1000	1000
Sodium benzoate	1000	1000
Sodium sulphite	1200	1400
Sodium sulphite acid	1150	1100
Sodium borate	1200	1400
Sodium carbonate	1400	1600

The egg albumen digested in neutral solution averages about 87 per cent. of the amount digested under similar conditions in acid solution. These figures show that the activity of the ferment is increased by acids or acid salts, irrespective of kind, mineral or organic. The apparent increase with alkaline salts is due to the solvent action of the alkali on the albumen. If the filtrate after digestion with alkaline salts is made acid and boiled, a second separation of dissolved but undigested egg albumen takes place and, introducing the correction, it is found that the results are abnormal, depending on the amount and kind of alkalinity. The corrected figures are always below 1000, showing that alkalinity retards the activity of the ferment.

The conclusions drawn from the experiments with pepsin and pancreatin were:

I. In an acid medium, the only preservatives or condiments used in these tests which retarded peptic or pancreatic digestion,

when in proportion of 1 : 400 or less, were salicylic acid, formaldehyde, smoke, condensed smoke and creosote. Of these, salicylic acid does not retard the digestion when present in proportion of 1 : 1000.

II. (*a*) In a neutral medium acid, preservatives and acid condiments increase the factor of digestibility, due to a change of the neutral medium to an acid one, and digestion proceeds normally as in the acid medium; (*b*) alkaline preservatives change the character of the medium and the results are abnormal, the action of the ferment being retarded.

III. In an alkaline medium, preservatives and condiments react abnormally, depending on the degree of alkalinity, the action of the ferments being retarded.

A similar series of experiments were made with colors, representing commercial products, submitted to me at various times for examination. The process was slightly changed, using acetic acid solutions and stirring the mixtures continuously during digestion, when using insoluble colors. No colored food product known to me contains over 1 ounce of color to 100 pounds of finished product, a proportion of 1 : 1600

Confectionery averages less than 1 part to 25,000 parts, and many other saccharine food products (sugar, glucose, etc.) less than 1 part of color to 1,000,000 parts of product. Not a single one of the colors tested in proportion of 1 part of color to 1600 parts of egg albumen, was found to affect the peptic nor pancreatic digestion *in vitro*.

The insoluble colors were washed to separate soluble impurities, and dried at a temperature below 100° C. All colors were either dissolved or suspended in water, and the requisite amount measured into the egg albumen solution. With the soluble colors the substance digested was figured as the difference between the egg albumen used and residue found. With insoluble colors the amount digested was taken as the difference between color and egg albumen used and residue found. Some of the insoluble colors were found to contain insoluble digestible fillers. Some of the soluble colors were found to contain soluble digestible fillers and a special correction is necessary. The results on such colors are omitted from the table and were not considered in the conclusions drawn.

The results obtained were as follows, the ratio of dry egg albu-

men (1600) to amount of color used (4, 8, 32 and 160) being placed at the head of the table.

WITH PEPSIN, IN ACETIC ACID SOLUTION.

	1600:4.	1600:8.	1600:32.	1600:160.
Check blanks	1000	1000	1000	1000
Mineral Colors.				
Prussian blue	1000	1000	1000	950
Ultramarine	950	900	850	800
Burnt sienna	950	900	850	800
Chrome yellow	950	900	800	700
Iron oxide (red)	1000	1000	950	900
Animal Color.				
Cochineal	1000	950	900	800
Vegetable Colors.				
Turmeric	1000	950	900	750
Annatto	1000	950	900	750
Indigo	1000	950	900	750
Cladonal red	1000	950	900	750
Synthetic Colors.				
Hofmann's violet, 3 B	1000	1000	1000	900
Paris violet	1000	1000	950	800
Bismarck brown	1000	1000	1000	950
Eosin	1000	1000	1000	950
Fuchsin	1000	1000	1000	900
Indigo	1000	1000	900	800
Congo red	1000	960	930	880
Naphthol yellow	1000	950	920	850
Ponceau, 2 R	970	930	900	800
Amaranth red	1000	960	930	900
Turmerine yellow	1000	1000	1000	950

From these results the conclusion is drawn that of the colors used only ultramarine, burnt sienna, chrome yellow and ponceau 2 R. affect artificial digestion with pepsin when used in quantities of 1 part of the color or less to 400 parts of the food

The results also indicate that the synthetic colors are less active than animal and mineral colors and not more active than vegetable colors. Vegetable and synthetic colors are directly digested by pepsin and by pancreatin, and when the amount of color exceeds 1 part to 200 a correction is necessary, increasing the factor of digestibility 10 to 40 points. If the direct digestion of a color by ferments is considered to show an actual food value, then it is found that no differences exist in food value between vegetable and synthetic colors. The apparent reduction in digestibility of the egg albumen with the mineral colors is due to the neutral-

izing or solvent action of the acid or to a direct combination of the color with the albumen. In neutral egg albumen solution the reduction in digestibility is so slight that same can be ignored and the mineral colors considered as inert matter. When the color exceeds 1 part to 400 parts of egg albumen, it is often impossible to wash the coagulated egg albumen free from color, and a slight error is thereby introduced, but so small that the same has been ignored. The action of pepsin and of pancreatin on the colors direct can be observed and determined by comparison, with or without dilution, of the filtrates from the coagulated egg albumen, comparing filtrates before and after treatment with ferments. In drawing conclusions from these experiments as to the actual efficiency of colors for food purposes it is shown that the synthetic colors are less active and retard the action of the digestive ferments less than any other class of colors, when used in quantities to give like shade of color to the food product, due to the fact that the coloring power of the synthetic colors is so much more intense, from 5 to 100 times as strong as that of vegetable colors, and the amount of color required therefore is reduced in the same proportion, exactly in the ratio of the comparative color intensities.

Artificial digestion experiments can only prove one point and are in themselves not sufficient to pass any color, preservative or condiment as to its suitability for food purposes. In connection with chemical analyses, physiological and feeding tests, they are of great value. If a color, preservative or condiment is found to retard or otherwise interfere with peptic or pancreatic artificial digestion, when used in quantity equal to or less than found in food products, such articles must be considered as not suitable for food purposes, irrespective of its source, whether natural or synthetic, whether of vegetable, animal or mineral origin.

903 POSTAL TELEGRAPH BUILDING,
CHICAGO, ILL.

A NEW BURETTE HOLDER.[1]

BY A. T. LINCOLN.

Received July 14, 1905.

THE INCONVENIENCE of the ordinary forms of clamps now in use for holding burettes, has been experienced by nearly every one

[1] This holder was exhibited before the Philadelphia meeting (December, 1904) of the American Chemical Society.