nels is greater than the increase in the weight of the kernels, resulting in a higher percentage of nitrogen, also, in the outer kernels, but there were frequent exceptions to this. It is apparent from these results that the kernels of the most desirable qualities came from the outer rows.

Briefly summarized, the results of our studies of the composition of the grain from different parts of a single head show that the best grains from the standpoint of weight of kernel, and nitrogen content, came from the outer grains of the spikelets in the middle of the spike. Whether these properties are capable of transmission to the succeeding generations can be determined only by experimental trial, which we are inaugurating.

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THE EFFECT OF COLORING MATTERS ON SOME OF THE DIGESTIVE ENZYMES[1].

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The following investigation was conducted under the supervision of the Department of Chemistry of The George Washington University. I wish to express my indebtedness to Drs. C. E. Monroe and T. M. Price for their suggestions and assistance.

A search through the literature indicates that there has been but little work done in the study of the effect of coloring matters on the digestive enzymes. However, that which has been accomplished by H. A. Weber[2], Edward Gudeman[3], A. J. Winogradow[4], and others has given results which are exceedingly interesting and important.

In the article by H. A. Weber, it is shown that oroline yellow effects the peptic digestion of fibrin, while saffoline, magenta and methyl orange effect the pancreatic digestion of this body.

Gudeman found that ultramarine, burnt sienna, chrome yellow and ponceau effected the artificial peptic digestion of egg albumen when used in such quantities as one part, or less, of the color to 400 parts of the food.

In the investigation conducted by A. J. Winogradow, he found that Safranine, Ponceau R. R., Azofuchsine, G., Orange II, Coeruline S., Phoxine, R. B. N., Iodeosine, Chrysaniline, Magdala red, Azoflavine,

[1] Presented before the meeting of the American Chemical Society at Toronto, Ontario, June 27-29, 1907; being a thesis submitted to The George Washington University for the Degree of Master of Science.

[2] On the Behavior of Coal-tar Color toward the Process of Digestion, by H. A. Weber, Am. Ch. J., 1896, XVIII, 1092-1096.

[3] Artificial Digestion Experiments, by Edward Gudeman, J. Am. Chem. Soc. 1905.

[4] The Influence of certain Coal-tar Dyes on the Digestion, by A. J. Winogradow. Z. Nahr. Genussm., 1903, VI, 589-592.

Benzopurpurine, and Cerise hindered the digestion of albumen by pepsin even when only a few milligrams of the colors were present; an amount corresponding to from 1:10 to 1:100 of the digestive solution. The action was almost inhibititive. The colors Quinoline yellow, Naphthylene green, Acid green, Iodine green, Acid azo-yellow T., Naphthol yellow, Aniline green, Primuline, Auramine O., Aniline orange. Martius yellow, and Metanil yellow interfered less than the first dyes, but in every case some effect was noticed.

As stated, in these experiments the investigator used his coloring matter in proportion from 1:10 to 1:100. This seems unpractical, as these colors are seldom used in such strengths in practice.

It was only after an extensive review of the work of these investigators that the following investigation was undertaken in order to ascertain the effect of other coloring matters which have not as yet been studied by artificial digestion.

In the investigations, the results of which are recorded herein, the effects of annatto, saffron, turmeric, cochineal, Bismarck brown and croceine scarlet 1-B were studied on the peptic digestion of fibrin, casein and egg albumen. There was also studied the effect of ground annatto seeds and oil yellow on the action of the fat decomposing enzyme lipase, when it was mixed with butter fat. The experiments were carried out under laboratory conditions and it is not claimed that they represent exactly the conditions which exist in an animal, or that the phenomena would occur in precisely the same manner in an animal system, but it is believed that from a consideration of these results we may infer, with a fair degree of accuracy, what would occur in an animal system, if the bodies studied were introduced into it.

The Effects of Coloring Matters on the Digestive Enzyme, Pepsin.

The extent to which the fibrin, casein, and egg albumen, used in these experiments, were digested, was ascertained by determining the amount of nitrogen in solution and the amount remaining in the residues after the materials used had stood in contact with the active ferments for two hours. The nitrogen in all samples was determined by Gunning's method, which is one of the most accurate methods known at the present day.

Preparation of Materials Used. The fibrin[2], was obtained by whipping freshly drawn blood with a suitable instrument, the fibrin being deposited as an elastic, stringy material, which was freed from adhering corpuscles by thorough washing and kneading. Fibrin, so obtained, is

[1] Gunning's Method for Nitrogen, Official Methods of Analysis, A. O. A. C. Bulletin 46, Bureau of Chemistry.

[2] Physiological Chemistry, by Charles E. Simon, M. D. Hand Book for Bio-Chemical Laboratory, by John A. Mandel.

still contaminated with serum-globulin and certain phosphorus-containing substances which have resulted from the decomposition of leucocytes. This serum-globulin was removed by separate washing and kneading in a 5 per cent. solution of common salt. Then when the fibrin had been further freed from sodium chloride it was extracted with alcohol and then with ether and finally preserved in a solution of equal parts of glycerol and water.

The casein was prepared by diluting 400 cc. of fat-free milk with four times its volume of water and acidifying with acetic acid to the extent of 0.75–1.0 pro mille. It was washed free of acid by decantation, filtered, shaken with anhydrous ether, again filtered and dried in a vacuum desiccator. After drying, it was pulverized and passed through a one millimeter sieve.

The egg albumen was obtained from fresh eggs by boiling them until the whites were hard. The coagulated whites were separated from the yolks and ground in a mortar, first with 95 per cent. alcohol, then with anhydrous ether. The ether was filtered off and the mass of coagulated egg albumen was placed in a vacuum desiccator and dried.

The annatto, saffron, turmeric and cochineal were prepared by placing a quantity of each coloring matter on a filter and treating each separately with boiled distilled water until practically the entire amount of the soluble coloring matter present had been extracted. This method required about a week for each coloring matter. Each of these extracts was evaporated to dryness and powdered. By this means the author avoided using material which was useless as coloring matter.

Mode of Procedure. In order to shorten the work so as to make it possible to handle the entire series of experiments on each substance at the same time it was found advantageous to use one half of a gram of the protein. This allowed the manipulator the opportunity to wash thoroughly each residue and still not have too large a volume of filtrate to handle.

The first protein studied in this series of experiments was fibrin, which is one of the coustituents of meat and meat products. Thus it is of interest to obtain some data as to the effect of these coloring matters on products which are eaten by man. These experiments were conducted under the most favorable conditions, and in order that there should be a uniformity of results, each series of experiments was completed at the same time, thus avoiding the possibility of having a slight lowering or rising of temperature in the different members of the series that would tend to decrease or increase the digestibility to a slight extent, for if this occurred it would be impossible to draw conclusions as to whether or not one coloring matter was more active than another.

Effect of Coloring Matters on the Peptic Digestion of Fibrin. The fibrin

was taken from the preserving fluid, placed on a filter and washed thoroughly with boiling distilled water until free from glycerol. Then it was placed in an oven and dried at 115°. When dried so that it could be handled, duplicate samples, 0.5 gram each, were weighed out for the total nitrogen determination. The remaining samples of 0.5 gram each were weighed out in Erlenmeyer flasks. In these respective flasks varying amounts of coloring matters were placed as shown in the table. After all the flasks were properly numbered and contained the desired constituents, fifty cubic centimeters of artificial gastric juice[1] were added and in each case the temperature was brought to 40°. Then they were placed in an incubator and kept at 40° for two hours, when they were removed and brought to a boil at once. They were immediately filtered and washed with boiled distilled water until the filtrates were no longer colored. When this stage was reached the respective residues of the samples were placed in properly labeled Kjeldahl flasks and the nitrogen determined.

The accompanying table will demonstrate the effect of some of the coloring matters used in different amounts of artificial gastric juice.

Fibrin	Per Cent. of Digestibility					
Ratio used	0	1:100	1:200	1:400	1:800	1:1600
Annatto	96.2	96.2	95.8	95.9	96.2	96.2
Saffron	96.2	85.9	94.7	96.2	96.2	96.2
Turmeric	96.2	61.2	69.2	80.3	94.0	96.2
Cochineal	96.2	56.4	96.1	95.7	96.2	96.2
Bismarck Brown	96.2	48.6	85.6	88.1	92.8	95.6
Croceine Scarlet 1-B	96.2	0.0	0.0	0.0	0.0	0.0

In this work the total amount of digested nitrogen in the filtrate was only determined where there was a considerable decrease in the per cent. of digestibility due to coloring matter. This was done to show that the determination of the digestibility of the substance can be made by determining it indirectly from the undigested nitrogen in the residue or by determining it directly from the digested nitrogen in the filtrate. As these two percentages of digestibility were approximately equal, the conclusion can readily be drawn that the determination of digestibility from the undigested nitrogen in the residue is just as accurate as the determination of the digestibility from the digested nitrogen in the filtrate.

The annatto when added to fibrin has no effect on the enzymic activity in any case.

The saffron, when in proportions of 1:100 diminishes the enzymic activity on fibrin, but it is seldom used in such a strength. When used in smaller quantities it is without effect.

The turmeric diminishes the enzymic activity when used in proportions

[1] Prepared by adding 10 mg. of pepsin to 50 cc. of a 0.2 per cent. solution of hydrochloric acid.

of 1:100, 1:200 and 1:400, while in the proportions of 1:800 and 1:1600, it is practically without effect.

The cochineal diminishes the enzymic activity when used in proportion of 1:100, but this strength is seldom used. Smaller quantities have no effect.

The Bismarck brown diminishes the enzymic activity in proportions of 1:100 and 1:200, but these are not practical, as the colors are too strong. Smaller quantities produce no effect.

The croceine scarlet 1-B stops the digestion of fibrin entirely. As it was seen by the determination of nitrogen in the residue that the fibrin absorbed some of the coloring matter and that none of the substance was digested, it is, therefore, fair to conclude that this coal-tar dye prevents the enzymic activity.

In practice the coal-tar dyes[1] are replacing the animal and vegetable coloring matters, for instance, croceine scarlet is replacing cochineal and the azo yellows are replacing vegetable coloring matters in butter.

Effect of Coloring Matters on the Peptic Digestion of Casein.—The 0.5 gram samples were weighed out in this series of experiments and digested in the same manner as with the fibrin. In each case the coloring matter was weighed out in the proportions as shown in the table.

Casein.	Per cent. of Digestibility.				
Ratio used..... 0	1:100	1:200	1:400	1:800	1:1600
Annato.....98.2	66.3	87.3	96.8	96.8	98.2
Saffron.....98.2	78.3	78.8	90.1	97.0	97.8
Turmeric.....98·2	31.6	73.2	79.0	87.1	87.2
Cochineal 98.2	71.8	86.1	87.3	98.1	98.1
Bismarck Brown...98.2	58.9	98.1	98.1	98.1	98.2
CroceineScarlet,1·B 98.2	0.0	0.0	18.7	56.6	70.0

From the results in this table it can be said that the coloring matters which appear to affect the peptic digestion of casein are saffron, turmeric, cochineal and croceine scarlet 1-B, but cochineal and saffron may be exempted from this classification, as these coloring matters are very seldom used in the proportion of 1:400, or larger, and in smaller proportions they have no effect on the enzymic activity.

Effect of Coloring Matters on the Peptic Digestion of Egg Albumen.—The same mode of procedure was followed with egg albumen as with fibrin and casein, as will be seen by the accompanying table.

Egg Albumen.	Per cent. of Digestibility.				
Ratio used 0	1:100	1:200	1:400	1:800	1:1600
Annatto.....54.0	30.8	47.1	47.0	46.9	47.3
Saffron.....54.0	15.0	34.8	36.6	50.9	53.4
Turmeric.....54.0	1.89	8.03	29.0	37.7	39.7
Cochineal 54.0	11.1	30.4	39 6	41.3	42.4
Bismarck Brown....54.0	10.6	27.5	30.3	30.3	33.3
Croceine Scarlet, 1-B 54.0	0.0	0.0	0.0	6.36	19.7

[1] The Scientific Development of the Coal-tar Color Industry, by Professor R. Meldola, R. C. S., F. I. C., J. Soc. Dyers Colorists, 1886.

The saffron is without effect except when it is used in the proportions of 1:100, 1:200 and 1:400.

The annatto, turmeric, cochineal, Bismarck brown and croceine scarlet 1-B diminish the enzymic activity in every case.

Conclusions.

From the study of the tables the following general conclusions may be drawn in regard to the effect of annatto, saffron, turmeric, cochineal, Bismarck brown and croceine scarlet 1-B, on the activity of peptic digestion of fibrin, casein and egg albumen:

(1) Annatto, when used in the given proportions, is without effect on the enzymic activity on fibrin while it diminishes the enzymic activity on egg albumen and casein.

(2) Saffron diminishes the enzymic activity on fibrin, casein and egg albumen when used in proportions varying from 1:100 to 1:400, while smaller quantities have no effect.

(3) Turmeric has no effect on the enzymic activity on fibrin when used in proportions of 1:800 or smaller, while with casein and egg albumen it diminishes the enzymic activity in each case.

(4) Cochineal and Bismarck brown diminish the enzymic activity on fibrin and casein when used in proportions varying from 1:100 to 1:400, while with egg albumen the enzymic activity is diminished throughout.

(5) Croceine scarlet 1-B prevents entirely the enzymic activity on fibrin, while with casein and egg albumen the enzymic activity is prevented when proportions of 1:100 to 1:200 are used. Smaller quantities of the same dye diminish the enzymic activity on casein and egg albumen.

Effect of Coloring Matters on the Hydrolysis of Butter Fat by Lipase.

Before giving the description of this series of experiments it will be of interest to refer to the relation of lipase to fat metabolism. The reversible action of lipase furnishes an explanation of fat absorption. Lipase is found in all tissues of the body that have been tested, notably in the liver, active mammary gland, blood, lymph and intestinal mucosa. The storing and translocation of fats may be called "lipogenesis," the process being analagous to "glycogenesis." Both phases of lipogenesis may be brought about by the enzyme, lipase, which is either fat forming or fat splitting, according to conditions.

Preparation of Materials Used.—The lipase was prepared from calf liver in the following manner:[1] Fifty grams of freshly chopped liver were weighed out and ground thoroughly in a mortar with water and sand until well macerated. The mass was allowed to digest at 40° C. for one day. It was then filtered and made up to 100 cc. so as to have a

[1] Concerning Lipase, the fat-splitting enzyme, and the reversibility of its action, by J. H. Kastle and A. S. Loevenhart, Am. Ch. J., 24, 491-525, 1900.

50 per cent. solution. A little chloroform and sodium carbonate were added to the solution so as to preserve its activity.

The butter fat was prepared from double cream by extracting the fat with anhydrous ether to which a little sodium carbonate had been added.

Mode of Procedure.—The first efforts were directed toward ascertaining the proportions in which each of the two coloring matters, annatto seed and oil yellow, were used for coloring butter. This was arrived at by mixing the coloring matters with lard in various proportions. With annatto seeds it was found that a good butter color can be obtained between the proportions of 1:500 to 1:1000. With oil yellow it was found that a good color can be obtained between 1:1000 to 1:10000. These proportions are not precisely used in practice for coloring butter, as this fat has very often a natural color, but if these colors when used in the given proportions do not affect the hydrolysis of the butter fat, they will not when used in smaller quantities.

In these experiments the intermediate proportions between these ratios were used. In each case one gram of the neutral butter fat was weighed out and the varying amounts of the coloring matters were added, as may be seen in the tables.

BUTTER FAT AND ANNATTO SEEDS.

Proportions used	0	1:500	1:1000	1:2000
Amount of butter fat used (grams)	1	1	1	1
Amount of annatto used (grams)	0.0	0.002	0.001	0.0005
Amount of lipase solution used (cc.)	2	2	2	2
Amount of water used (cc.)	10	10	10	10
Length of digestion (hours)	7	7	7	7
Amount of N/20 HCl used (cc.)	0	0	0	0
Amount of N/20 NaOH used (cc.)	0.4	0.5	1.5	1.6

BUTTER FAT AND OIL YELLOW.

Proportions used	0	1:1000	1:2000	1:5000	1:10000
Amount of butter fat used (grams)	1	1	1	1	1
Amount of oil yellow used (grams)	0.0	0.001	0.0005	0.0002	0.0001
Amount of lipase solution used (cc.)	2	2	2	2	2
Amount of water used (cc.)	10	10	10	10	10
Length of digestion (hours)	6	6	6	6	6
Amount of N/20 HCl used (cc.)	0	0	0	0	0
Amount of N/20 NaOH used (cc.)	0.5	1.8	1.2	1.0	1.0

Conclusions.

From the results shown in the tables it may be seen that there were no retarding effects caused by the coloring matters, annatto or oil yellow. As a matter of fact, each one aided the hydrolysis of the butter fat. There is no explanation to give for this increase of hydrolysis of the butter fat except that it might be suggested that the coloring matters contain some free acid. But this is not justified, as checks were made in each case. The only reason that can be given is that the coloring matters contain some lipolytically active substance as a part of their composition.[1]

[1] On the Action of Lipase, by Alonzo E. Taylor, J. Biol. Chem., 8, 87-104, 1906.