drying in an air-bath at 101° or 102° (on account of the tenacity with which the humus retains water), the dish with the humus materials is weighed, then ignited, and weighed again. The loss of weight is calculated as humus. The humus extract may be either black or brown.

Nitrogen Content of the Humus.—Either five or ten grams of soil are first treated with dilute hydrochloric acid, in the same way as in the humus determination. If the soil contains only a small amount of organic material, fifteen or twenty grams should be taken for this determination. After treatment with acid the soil is extracted with a three per cent. solution of sodium hydroxide in the same way as for the ammonia extraction. Two hundred cc. of the filtered solution are evaporated nearly to dryness in a Kjeldahl distillation flask, after first making the solution acid with sulphuric acid. The nitrogen determination is then completed in the usual way.

THE ACTION OF CERTAIN BODIES ON THE DIGESTIVE FERMENTS.

BY FRANK D. SIMONS.

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VERY little attention seems to have been given to the study of the action of some of our common coloring matters, flavoring extracts, and preservatives on the digestive ferments. The object of my work, therefore, has been to test, from a physiological standpoint, the deportment of members of the above three classes of bodies towards digestion.

As far as my searches have extended, I have been able to find but very little literature bearing on this subject. Professor Chittenden, of the Sheffield Scientific School, has furnished many valuable contributions to our knowledge of digestion. He includes in a list of bodies antagonistic to the peptic ferment, potassium permanganate, borax, ammonia-alum, sodium salicylate, quinine, and the salts of nearly all of the alkaloids.

Dr. H. A. Weber[1] investigated the behavior of three coal-tar coloring matters (*viz.*, oroline yellow, saffoline, and magenta) towards digestion. Although these bodies have been included in the list of harmless colors, yet he found that each one, even

[1] This Journal, 18, 1092, *1896*.

when taken in a very minute quantity, arrested digestion, some of them that of the stomach, others that of the intestines, though in no case did he find that any one substance alone had a retarding effect on both peptic and pancreatic digestion. From his results then, it would seem that these substances are very harmful, inasmuch as they interfere seriously with the natural functions of the digestive organs, or more correctly speaking, with their secretions.

As an example of the possible danger from the indiscriminate use of these comparatively unknown (at least so far as concerns their relations toward digestion) colors, we quote the results of Mr. Weber's experiments with oroline yellow on stomachic digestion:

"This color was one of a series employed in the coloring of confectionery, and was found to be what is known in the trade as acid yellow or fast yellow, and is a mixture of sodium amidoazobenzenedisulphonate with sodium amidoazobenzenemonosulphonate.

	Amount of color. Gram.	Amount of fibrin. Gram.	Amount of pepsin. Gram.	Duration of experiment. Hours.	Amount of fibrin dissolved. Gram.
1.....	0.0	1	0.020	3	1.00
2.....	1.0	1	0.020	3	0.10
3.....	0.5	1	0.020	3	0.12
4.....	0.25	1	0.020	3	0.22
5	0.125	1	0.020	3	0.35
6.....	0.062	1	0.020	3	0.73

From this it will be seen that even in test No. 6, where the color employed amounted to only one part in 1,600 parts of the solution, the presence of the color had still a depressing effect. On the whole it must be conceded that this color has a marked and injurious effect on peptic digestion."

Now when we see what a small portion of the poison (I call it so, for undoubtedly it is) will retard digestion, the only natural inference, which one might draw, would be that it is antagonistic to the normal action of the organs. However, that is a question for the physician rather than for the chemist, for while the latter performs the experiments, the former applies their results.

In carrying on an artificial process of digestion the substance usually employed to be digested is freshly prepared blood fibrin or purified and coagulated egg-albumin.

To prepare the former the blood fresh from the artery must be well whipped, and the fibrin thus obtained washed quickly and thoroughly with water. When washed well, it should be perfectly white, showing no tint of red. While the freshly prepared fibrin is always better for the tests, yet, as the investigator is seldom in a position where this can be accomplished without some difficulty and loss of time, it may be preserved to good advantage in alcohol; in this manner it can be kept from decomposition for some time. Before using, however, it must be washed entirely free from the spirits, then dried, and the amount of material desired weighed.

To prepare coagulated egg-albumin treat the whites of freshly laid eggs with an equal volume of water, shake well, and exactly neutralize with hydrochloric acid. Then filter until the filtrate is comparatively clear, and pour into about an equal volume of boiling water. Render faintly acid with acetic acid[1] and heat to vigorous boiling. The precipitate is now filtered off, and washed several times with boiling water. It is then ready for use, and like fibrin may be preserved to good advantage in alcohol. When the process has been rightly carried on, the albumin should be perfectly white, firm, and capable of being easily washed and dried. There should be no sticky feeling when held in the hands, and when such is the case, it must be submitted to further boiling, and perhaps even to a slight neutralization.

I found the albumin to be better for my experiments than the fibrin, as it is more delicate in reaction; that is, it is a little more difficult to digest than the fibrin, thus showing retarding effects to a finer degree. However, when the latter substance is employed in the process, the work must not be delayed; in other words, after digestion has continued the desired number of hours, the undissolved fibrin should be quickly filtered, washed, and weighed; for fibrin decomposes rather quickly in the air, and by that means some of the undissolved substance might be lost, which, of course, would lead to erroneous results.

As an artificial gastric juice the following proportion was used: Two-tenths gram of purified pepsin dissolved in 10.2 cc. hydrochloric acid (sp. gr. 1.14), and this then made up to a liter.

[1] Care must be taken to add only just enough of the reagent to render the liquid very faintly acid. It must be remembered that albumin is very soluble in a slight excess of the acid.

Gastric juice prepared by simple extraction from pigs' stomachs with hydrochloric acid is not only not constant, but also often faulty in its action. These extracts contain slime-like albuminous bodies (not further understood) which contaminate the digestion. In order to avoid this, Kühne[1] recommends a juice prepared in the following manner:

"The prepared mucous membrane of the pig's stomach is digested six days at 40° C. with seven times its quantity of a five-tenths per cent. hydrochloric acid solution. Saturate now directly with ammonium sulphate; collect the precipitate as completely as possible, and wash with water. Dissolve in digestion hydrochloric acid (five-tenths per cent.) five times the amount of the membrane taken, which contains (0.25 per cent.) thyme oil, and again digest one day at 40° C. Saturate now with ammonium sulphate. The residue ('purified pepsin') is used for the digestion by suspending it in digestion hydrochloric acid."

To avoid the difficulty and loss of time attending the preparation of pepsin, the powdered article from the laboratories of Armour & Co. is recommended. If this extract is used, the juice should be made up in the above proportion: *viz.*, two-tenths gram pepsin, and 10.2 cc. hydrochloric acid (sp. gr. 1.14) dissolved in one liter of water.

For the intestinal digestion the artificial pancreatic juice was made by dissolving a mixture of three grams pancreatine (procured of Armour & Co.) and fifteen grams sodium bicarbonate in one liter of water. The pancreatic solution may, however, be prepared in the following manner:

After removing the fat from the pancreas of a beef, divide that organ finely (with a sausage mill or chopping knife). Triturate with fine, clean sand, and let stand twenty-four to thirty-six hours at 14°. Now treat the mixture with glycerol and water, employing for every 400 grams pancreas one liter of glycerol (sp. gr. 1.23) and one liter of water. After the lapse of three days press in a filter press, then through filter paper, and to the filtrate add five cc. chloroform (to prevent bacterial changes). Preserve in a cool place. When used, one gram sodium bicarbonate is added to each 100 cc. of the trypsin solution.

[1] *Zeit. f. Biol.*, **22**, 426-428.

In carrying on my experiments a control test, consisting of a mixture of 100 cc. of the digestive liquid and one gram of the substance to be digested (blood fibrin or egg-albumin), was placed in a 150 cc. flask, immersed in a water-bath, and subjected to a temperature of from 38° to 40° C., until complete digestion was manifest. Then, at the same time, and under the same conditions, similar mixtures, but with the addition of varying quantities of the body to be tested, were subjected to a like treatment. When the digestion of the control test was completed, all of the tests were cooled to the temperature of the laboratory, and then filtered and washed through tared filter papers. After drying, they were weighed, using the control filter paper as a control weight, and the amount of the undigested material thus calculated.

It can be seen from the method of procedure that my results are, to a certain extent, approximate. They are, however, accurate enough to show distinctively the behavior of the substances tested toward the digestive ferments.

I have grouped the substances with which I worked under the following heads, and this order will be adhered to in their discussion:

I. Digestion retarded even after prolonged treatment:

a. *Peptic:*

Picric acid.
Tropaeolin ooo.
Metanil yellow.

b. *Pancreatic:*

Bismark brown.
Cinnamon.
Formol.

II. Digestion ensues only after prolonged treatment:

Peptic:

Salicylic acid.
Oil of wintergreen.

III. Digestion progresses normally:

Peptic and Pancreatic:

Essence of peppermint.
Chrysoidine.
Safranine.
Methylene blue.

Picric Acid, $C_6H_2(NO_2)_3OH$.

The following results were obtained:

Peptic Digestion.

Test.	Amount of substance. Gram.	Amount of albumin. Gram.	Duration of experiment. Hours.	Material not digested. Gram.
1	0.00	1	6	0.00
2	0.60	1	6	1.00
3	0.30	1	6	0.92
4	0.20	1	6	0.76
5	0.10	1	6	0.55
6	0.06	1	6	0.32

Pancreatic Digestion.

1	0.00	1	12	0.00
2	0.60	1	12	0.00
3	0.30	1	12	0.00
4	0.20	1	12	0.00
5	0.10	1	12	0.00
6	0.06	1	12	0.00

From the above results it will be seen that while the acid had a distinctively depressing effect on the peptic ferment—as is shown even in test No. 6, where the amount of the acid was only one part in 1,666 parts of the solution—yet its deportment toward the pancreatic ferment was perfectly normal.

The presence or absence of digested albumin in the solution may be proved by the addition of a solution of tannin, which reagent precipitates albuminous bodies.

Tropaeolin ooo No. 1, $NaSO_3.C_6H_4.N:N.C_{10}H_6OH$.

Peptic Digestion.

Test.	Amount of substance. Gram.	Amount of albumin. Gram.	Duration of experiment. Hours.	Material not digested. Gram.
1	0.00	1	6	0.00
2	0.60	1	6	1.00
3	0.30	1	6	0.96
4	0.20	1	6	0.84
5	0.10	1	6	0.70
6	0.06	1	6	0.48

Thus it will be seen that this body has a very deleterious effect in the peptic ferment.

The color was found to have no retarding effect on the intestinal digestion, the albumin in all of the tests dissolving completely at the expiration of twelve hours.

METANIL YELLOW, $NaSO_3.C_6H_4.N:N.C_6H_4.NH.C_6H_5$.

PEPTIC DIGESTION.

Test.	Amount of substance.	Amount of albumin.	Duration of experiment.	Material not digested.
	Gram.	Gram.	Hours.	Grams.
1	0.00	1	6	0.00
2	0.60	1	6	1.00
3	0.30	1	6	0.93
4	0.20	1	6	0.52
5	0.10	1	6	0.41
6	0.06	1	6	0.32

This color, like the two preceding, has a markedly depressing effect on peptic ferment, but none at all on the pancreatic.

BISMARK BROWN, $C_6H_4(NH_2)—N—N—C_6H_3(NH_2).2HCl$.

PANCREATIC DIGESTION.

Test.	Amount of substance.	Amount of albumin.	Duration of experiment.	Material not digested.
	Gram.	Gram.	Hours.	Gram.
1	0.00	1	12	0.00
2	0.60	1	12	1.00
3	0.30	1	12	0.88
4	0.20	1	12	0.63
5	0.10	1	12	0.50
6	0.06	1	12	0.26

This color neither prevented nor delayed the peptic digestion.

The addition of Bismark brown to the pancreatic juice produced a heavy precipitate, consisting of the free color base. Therefore, control tests had to be made for these experiments. This was done by subjecting the same amount of the color to treatment similar to that of the test then being made—but without the addition of the substance to be digested—and after filtration, by using this precipitate and filter paper as a control weight.

In order to show that the depressing action on the ferment was caused by the Bismark brown itself, rather than by any mechanical influence which might have been produced by the presence of the precipitate in the solution, another test was made, wherein the precipitate was filtered off before the addition of the albumin, and the clear filtrate used as the digestion fluid. The result of this experiment was that the action of the ferment

was interfered with to the same extent as in the tests in which the precipitate was present.

CINNAMON.

The spirit of essence of cinnamon, prescribed by the U. S. Pharmacpoeia, is made up in the following proportions: Ten parts (one oz. av.) oil of cinnamon, and ninety parts alcohol.

The essence, prepared as given above, was used in the following experiments:

PANCREATIC DIGESTION.

Test.	Amount of substance.	Amount of albumin.	Duration of experiment.	Material not digested.
	Drops.	Gram.	Hours.	Gram.
1	00.0	1	12	0.00
2	30.0	1	12	1.00
3	20.0	1	12	0.90
4	10.0	1	12	0.68
5	5.0	1	12	0.24

No disturbance of the action of the peptic ferment by the introduction of this substance was produced, the digestion of the albumin in all of the experiments keeping pace with that of the control test.

FORMOL.[1]

A forty per cent. solution of formic aldehyde was used in these experiments.

PANCREATIC DIGESTION.

Test.	Amount of substance.	Amount of albumin.	Duration of experiment.	Material not digested.
	Drops.	Gram.	Hours.	Gram.
1	00.0	1	12	0.00
2	35.0	1	12	1.00
3	25.0	1	12	0.82
4	15.0	1	12	0.60
5	10.0	1	12	0.44
6	5.0	1	12	0.18

As in the case of the two preceding substances, formol had no apparent effect on peptic digestion.

It is seen from the above tests that this preservative has a very depressing action on the pancreatic ferment, even one part in 2,000 parts of the solution (test No. 6) being sufficient to distinctively retard digestion.

[1] Formula (?) $C(H)_2(OH)_2$. *Cf. Chem. Ztg.*, *1890*, 1408.

Salicylic Acid, $C_6H_4(OH).COOH$.

This body was found to retard the action of the peptic ferment, inasmuch as it considerably prolonged the time for complete digestion. Therefore, I made two series of tests: In the first, the amount of undissolved albumin at the completion of the digestion of the check test was recorded; in the second, the time necessary for complete digestion was noted. Thus:

Peptic Digestion.

Test.	Amount of substance. Gram.	Amount of albumin. Gram.	Duration of experiment. Hours.	Material not digested. Gram.
1	0.00	1	6	0.00
2	0.60	1	6	0.85
3	0.30	1	6	0.56
4	0.20	1	6	0.34
5	0.10	1	6	0.25
6	0.06	1	6	0.10
1	0.00	1	6	0.00
2	0.20	1	11	0.00
3	0.10	1	$9\frac{1}{2}$	0.00
4	0.06	1	7	0.00

This acid is generally conceded to have a retarding action on digestion;[1] my reason for experimenting with it, however, was that I wished to compare the results with those of the following substance, wintergreen, the active principle of which is methyl salicylate.

Wintergreen.

For the following tests I used a preparation procured at a pharmacy, and recommended as the natural oil of wintergreen.

As in my experiments with salicylic acid, I conducted here two series of tests—first, the amount of material undissolved at the completion of the digestion of the control test was recorded, and second, the time for complete digestion was noted.

Peptic Digestion.

Test.	Amount of substance. Drops.	Amount of albumin. Gram.	Duration of experiment. Hours.	Material not digested. Gram.
1	00.0	1	6	0.00
2	30.0	1	6	0.74
3	20.0	1	6	0.58
4	15.0	1	6	0.42
5	10.0	1	6	0.38
6	5.0	1	6	0.16

[1] Olaf Hammarsten: Physiological Chemistry, *1893*.

Test.	Amount of substance. Drops.	Amount of albumin. Gram.	Duration of experiment. Hours.	Material not digested. Gram.
1	00.0	1	6	0.00
2	15.0	1	12	0.00
3	10.0	1	11	0.00
4	5.0	1	8	0.00

By comparing the results of the experiments of salicylic acid and wintergreen a striking similarity of action on the peptic ferment will be noticed.

Wintergreen, as was expected from the behavior of salicylic acid, had no influence on the pancreatic digestion.

PEPPERMINT.

The essence, prepared according to the U. S. Pharmacopeia, *viz.*, oil of peppermint one fluid ounce, rectified spirits four fluid ounces, was used in my experiments with this body.

The action of peppermint on both the peptic and pancreatic ferments was found to be practically *nil.* For this reason it is useless to tabulate the different tests which were made. It is enough to say that the albumin in all cases, where the amount of the essence introduced varied from ten drops to three cc., digested in a perfectly normal manner.; in other words, complete solution had taken place at the end of six hours for peptic and twelve hours for pancreatic.

The three coloring matters, chrysoidine, saffranine, and methylene blue, like the essence of peppermint, had no apparent effect on the action of either of the ferments. In all of the tests made, with the amount of the color introduced varying from 0.06 to 1.00 gram, the albumin was found to be completely dissolved at the end of the allotted time.

Summing up my experiments now, it will be seen that of the twelve substances with which I worked, three (picric acid, tropaeolin ooo, and metanil yellow) distinctly arrested peptic digestion, three (Bismark brown, cinnamon, and formol) pancreatic digestion, two (salicylic acid and wintergreen) partially arrested peptic digestion, and four (peppermint, chrysoidine, saffranine, and methylene blue) had no apparent effect on either of the ferments.

It is probably assuming too much for one to assert, or to state as having proved, that certain bodies completely arrest digestion. This will be seen at once if we consider that these artificial processes of digestion probably differ in a great many respects from those natural processes occurring in the stomach and intestines.

An answer to the question, "Does a substance seriously arrest digestion?" is perhaps not, strictly speaking, to be given in general, because the results obtained hold good only for the conditions under which the experiments were conducted; in other words, the neutralization capacity of the products of digestion must be taken into consideration. However, if we can show that some substances by themselves entirely, or nearly so, neutralize the action of the digestive ferments, we must naturally, and justly too, I think, infer that they have a very decided unwholesome effect on some of nature's functions. For, even if we assume that as fast as one portion of the digestive fluid is neutralized more is supplied until the right proportion for normal digestion is reached, one must see what a drain these inimical substances cause the organs to be subjected to. The organs supplying the fluids must be subjected to double, triple, or perhaps even more, work than that which normally they are required to perform; and the food too remains in the stomach and intestines a much longer period of time than usual, which fact alone would prove the arresting power of these foreign bodies.

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[CONTRIBUTION FROM THE LABORATORY OF ANALYTICAL CHEMISTRY, UNIVERSITY OF MICHIGAN.]

A PRELIMINARY THERMOCHEMICAL STUDY OF IRON AND STEEL.

BY E. D. CAMPBELL AND FIRMAN THOMPSON.

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ALTHOUGH much work has been done upon the relation between the ultimate chemical composition and the mechanical and magnetic properties of iron and steel, very little has been done in the way of studying the question from a thermochemical standpoint.

In 1875 Troost and Hautefeuille[1] determined the amount of

[1] *Compt. rend.*, 80, 964.