A STUDY OF THE EFFECT OF COOKING ON THE DIGESTIBILITY OF WHOLE EGG PROTEIN

Thesis for the Degree of M. S.

ROBERTA HERSHEY

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THESIS

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By

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I N D E X

A STUDY OF THE EFFECT OF COOKING ON THE DIGESTIBILITY OF WHOLE EGG PROTEIN.

1. PURPOSE

The purpose of this study was to determine the effect of different methods of preparation upon the rate and completeness of digestibility and upon the assimilation and use of the protein of whole egg.

2. REVIEW OF LITERATURE.

Many articles on the digestibility of egg as influenced by cooking are included in the literature on the subject. However, for the most part, the work has been directed toward the determination of the digestibility of egg white due, no doubt, to the wide spread use of raw egg white in diets for invalids. Most of the work reported, unless limited to yolk or white of egg, is confined either to gastric or to tryptic digestion.

Dr. H. C. Sherman in his "Food Products" (1) says, "The digestibility of eggs has been studied experimentally, but not in such detail as with some other articles of food. ********** It is probable that eggs 'soft cooked' at a temperature below that of boiling water are the most readily and rapidly digested, but the ultimate

thoroughness of digestion does not seem to be greatly influenced by the method of cooking."

Some of the earliest work on digestibility of egg is reported with Dr. Beaumont's classic experiments on Alexis St. Martin (2). Through the permanent opening in the stomach of his subject, Dr. Beaumont observed the following:

Method of Cooking	Time of Leaving Stomach
Hard boiled eggs	3½ hours
Fried eggs	3½ hours
Soft boiled eggs	3 hours
Raw eggs	2 hours
Raw (whipped) eggs	1½ hours

He also used methods of artificial digestion and found the relation between types of preparation the same, but the

actual time longer. He omitted entirely digestion in the intestine.

Rubner (2) tested hard boiled eggs with a healthy man as a subject. He found 97% of the egg protein to be digested.

Langworthy in a Farmer's Bulletin on "Eggs" (2) reviews the results of some artificial peptic digestion experiments and nitrogen balance experiments carried on by the Minnesota Experiment Station. By artificial digestion with a pepsin solution, they found that an egg boiled 3 minutes and digested for 5 hours left 8.3% undigested protein, a 20 minute boiled egg left 4.1% undigested protein. However, eggs soft cooked for either 5 or 10 minutes were entirely digested in the same period. In a nitrogen balance

experiment on one man, they found 90% of the total nitrogen of a diet of cream, milk, potato and egg to be digested.

Jorissenne (2) concluded from his work that mastication was of prime importance in the digestibility of eggs.

Tikhivinski (2) at St. Petersburg conducted some more extensive experiments on a man, feeding first a diet of "hard-boiled" eggs, bread and meat, and then one of "soft-boiled" eggs, bread and meat. He concluded that eggs prepared by both methods were equally digestible. However he raised the question whether, when two foods are eaten together, if the digestibility of each may not be changed.

Linossier (3) found that gastric digestion lessened with the cooking of albumin, and Talarico (4) reported that tryptic digestion of egg albumin increased with the temperature and duration of heating.

Bateman (5) concluded during his series of experiments on dogs and rats that raw egg white was only 50 - 70% utilized, while cooked egg white was 90%. In every case he found that the raw whites caused intestinal disturbances, which were not observed while cooked whites were being fed. The egg whites, making up 20, 40, and 60% of the diets, were mixed with cracker meal and lard. The yolk of the egg, Bateman considers to be well utilized. Data was also collected from a number of people who consumed large quantities of raw and cooked eggs, the results

of which bore out the conclusions reached on the animal experiments. However, no information was given in the paper concerning the amount of egg eaten, the exact number of people on the experiment, or the per cent of the egg utilized.

Hawk and his co-workers (6) at Jefferson Medical College did a very comprehensive piece of work on medical students. They tested eggs prepared in many ways, analyzing the stomach contents at definite intervals after the egg had been eaten. In brief, the following results were obtained:

1. Raw egg white left stomach most rapidly.
2. Raw egg yolk required much longer than white.
3. Hard-cooked eggs took longer then soft.

4. Scrambled eggs took longer than boiled.

5. Poached, shirred, and soft-cooked eggs were most rapid.

Rose & Mac Leod (7 & 8) carried on some experiments with raw white of egg on six human subjects and found raw whites to be 80% digestible and cooked whites 86%. They found absorption to be better when the eggs were beaten light.

Some very recent reports from German investigators (9) contribute interesting additions to this problem. Sheunert and Wagner after experimenting on rats, conclude that the difference in nutritive value between raw and hard-boiled egg yolks is insignificant. Friedberger and Abraham found that animals on an egg diet suffered toxic results, which were nullified if the eggs were boiled 20 to 30 minutes.

Mitchell & Carman (10 and 11) report

whole eggs to be 100% digestible and to have a biological value, according to Mitchell's formula, of 94. In their experiments, the eggs were dried whole and the fat extracted with ether, they were then fed to rats and complete metabolism data was obtained.

An analysis of these articles, shows that more work has been done on the rate of digestibility than upon its completeness. The experiments of Drs. Beaumont and Hawk are definite results upon the speed with which eggs leave the stomach, but they give no indication of the percentage of egg ultimately digested since peptic digestion is only one step in the whole process. The artificial digestion experiments give only percentages of protein digested in a given period of time, and, while they are excellent measurements of the relative ease of digestion, they

again do not show the actual biological value of the food tested. Reports on percentages of digestibility obtained by feeding cooked eggs to human subjects vary from 90% to 97% with no very definite control of the method of cooking mentioned.

This work was planned to study further the influence of different methods of preparation upon the ease and completeness of digestibility and to, if possible, see if cooking methods would alter the actual biological value.

3. PROCEDURE.

The eggs used in these experiments were of a constant variety, being supplied, strictly fresh, by the College Poultry Department. They were selected, large, white eggs. Four methods of preparation were chosen for investigation,- two, supposedly "easy of digestion," the raw and soft cooked, and two "difficult of digestion," the scrambled and hard boiled. The raw eggs were whipped with a Dover egg beater, care being taken to incorporate a minimum amount of air. The hard-boiled eggs were placed in boiling water for 10 minutes. The scrambled eggs were first beaten and then cooked in butter in the proportion of 5 gm. butter to each egg. They were stirred continuously and heated until the protein was coagulated. The soft-cooked eggs were kept in water just below the boiling point for 10 minutes.

In order to secure uniform samples, the cooked eggs were run through a fine-meshed sieve several times.

The experimental work was divided into two parts: determination of the percentage of amino nitrogen formed in artificial digestion experiments, and nitrogen metabolism experiments on white rats. The former were time controlled and designed to determine the relative rate of digestion, while the latter would, of course, show completeness of digestion.

THE AMINO NITROGEN METHOD

The amino nitrogen method was essentially that of Waterman and Jones (12). About 1.5 grams of egg was suspended in 25 cc. of 0.1 N.HCL, 25cc of 0.2% solution of pepsin in 0.1 N.HCL was added and the mixture

digested at 37° C. for 1½ hours. Peptic digestion was brought to an end by the addition of 5 cc.N. NaOH and tryptic digestion started immediately with 5 cc. of a 6.0% solution of trypsin in 0.1 N.NaOH. The mixture was digested at 37° for 2½ hours and then the enzymes were destroyed by heating to 80° for 5 minutes. The solution was cooled, made up to 100 cc., filtered if necessary, and 10 cc. aliquots taken for the determination of amino nitrogen by Van Slyke's (13) method.

A blank determination was run with each set of triplicate experiments. The volume of nitrogen obtained from this blank was subtracted from that obtained with the egg. Undoubtedly self-digestion is greater in a blank where no substrate is present, than it is in digests containing a protein

sample, however, since identical technique was used in each case, the relative results would be the same.

Total nitrogen was determined by the Kjeldahl method and the percentage of amino nitrogen calculated. The results of these experiments are in Table 1. Each figure is an average of three Kjeldahl determinations or three amino nitrogen determinations. Considerable trouble was encountered in obtaining close checks because of difficulty in sampling, since it was necessary to use very small amounts in order that all the egg be in contact with the digestive enzymes.

There was quite a wide variation in the nitrogen content of the eggs as shown in Table 1. The nitrogen varied from 2.06% to 2.72%,- a

percentage variation of 27. These variations, while they are within the range given in the bulletin on the chemical composition of food materials (14), are all the more surprising in view of the fact that the eggs were from the same flock of hens which were receiving a constant ration.

B. Metabolism Method.

Four healthy, full-grown, male rats from the laboratory stock colony were chosen for the metabolism studies. Animals which had attained their full growth were used so that the problem would not be complicated by varying needs of protein for growth, but would be based upon the maintenance requirement.

The basal diet consisted of 96% cornstarch and 4% McCollum's salt mixture #185, to which was added daily weighed, 400 mg. portions of dried yeast. The nitrogen contained in the yeast did not enter into the calculations since the amount was exactly the same for each period, and any slight change it might cause would make no difference in the relative results for the five periods.

The eggs were fed in such amounts as to give an approximate 10% protein level, Mitchell (15) having shown that

the biological value of a protein decreases as the percentage of protein fed increases. The raw, hard boiled and soft cooked eggs were supplied in 8 gm. portions daily, while the scrambled egg was given in 8.6 gm. amounts to make up the protein replaced by the added fat. The eggs were cooked and placed in a tightly covered jar and kept in the ice box. In this way two samples were sufficient for the six-day period. Each sample was analyzed for total nitrogen and the three-day quota of nitrogen calculated. The two results were then added together and an average taken for the daily nitrogen supplied.

No basal ration was given in any case until all the egg had been eaten and after that, plenty of food was kept in the cages. It was necessary to keep the rats in their individual cages on an egg diet for some time before the experimental period to accustom them to

the flavor of eggs and to the somewhat unpalatable basal diet. Distilled water was given in inverted tubes ad libitum.

The collection method was the one of H. H. Mitchell (16) modified to suit conditions in our laboratory. The rats were placed in inverted circular wire cages, the top of the cage thus forming a raised screen bottom. The pans in which the cages were placed were covered with filter paper to absorb the urine. The food was placed in a cup and suspended in a metal ring fastened in an opening at the side of the cage. It was not possible to keep an accurate record of the amount of the basal diet consumed, because rats have a habit of eating food with their fore feet and scatter much of it. The portion that fell through the raised screen became contaminated with

urine and therefore had to be included in the washings later. Since the ration contained no nitrogen, this had no effect upon the determination of the nitrogen content of the excreta.

The experimental period consisted of a three-day preliminary period, since it has been shown that the endogenous level of urinary excretion in rats is reached in from one to four days on a nitrogen-free diet (16), and a six-day collection period. During the latter, the urine and feces were collected on alternate days. The rats were weighed at the beginning and at the end of each period.

The dish, paper, and raised screen bottom were washed with 300 - 400 cc. of boiling water

acidified with sulphuric acid using 10-12 washings. They were kept in the ice box over night and made up to 500 cc. volume the next day at ice box temperature. Six-day composite samples of the washings were kept and analyses for total nitrogen made by the Kjeldahl method.

The feces were preserved in 10% sulphuric acid, digested until a homogeneous suspension was obtained and made up to 500 cc. volume. Aliquots were taken for nitrogen analysis, which was also done by the Kjeldahl method.

4. DISCUSSION.

A. Artificial Digestion Experiments.

The data on these experiments is given in Table 1. These figures represent the checks obtained from some seventy triplicate determinations of amino and Kjeldahl nitrogen. It was especially difficult to obtain checks on the amino nitrogen determinations since there were so many variables outside of the possible variation in the way the eggs themselves might react. Enzyme preparations are not of constant strength and are known to deteriorate upon aging. Three separate bottles of powdered trypsin and two of pepsin were used during these experiments, which might account for some of the day-to-day variations.

A clumping of the egg during the digestion period was often noted in the cooked samples. This would obviously

lessen the degree of digestion by reducing the surface of the egg in contact with the digestive solutions. It is quite possible that heating for 5 minutes at 80° C. might not, in every case, destroy all the enzyme so that slight digestion might go on after the time record had been taken. This explanation is somewhat substantiated by the fact that several times the last sample of a group to be used for analysis in Van Slyke's apparatus gave the hightest percentage of amino nitrogen.

The percentages given in the table, with one exception, are averages of three determinations of 5% difference or less. In many cases the percentage difference is only 1 or 0.8. It would seem, consequently, that the data given is indicative of the actual behavior of egg protein during artificial time-

controlled digestion.

From these figures one would conclude that "soft cooked" eggs, cocked for 10 mimutes in water just below the boiling point, are more rapidly (and perhaps more easily) digested than the others, an average of 37.7% of the total nitrogen having been changed to amino nitrogen during the four-hour digestion period. Hard boiled eggs seem to be the next most rapidly digested, 30.5% amino nitrogen having been obtained in the same period of time.

The result with raw eggs is the surprising factor, since they are popularly thought to be most easily and rapidly digested. There was only 3.3% more amino nitrogen after four hour's digestion than was produced by scrambled eggs, which were well coated with fat, a substance

which has long been known to slow up processes of digestion.

The greatest difference between the various samples tested is shown by the 7.2% increase in the amounts of amino nitrogen produced by soft cooked over hard boiled eggs. There was a difference of 2.4% between the hard boiled and raw eggs, with one of 3.3%, already referred to, between the raw and scrambled ones. It is quite probable that in normal persons these small variations in the rapidity of digestion would be insignificant when the eggs were taken as a part of a mixed diet.

Table 1.

Percentage of Protein Digested by Artificial Digestion.

Method of Cooking	Total Nitrogen per cent	Amino Nitrogen per cent	Nitrogen Digested per cent
Raw	2.51	.718	28.6
	2.30	.647	28.1
	2.35	.653	27.6
Av.			28.1
Hard boiled	2.26	.647	30.0
	2.19	.715	32.6
	2.72	.753	27.6
	2.13	.678	31.8
Av.			30.5
Scrambled	2.31	.585	25.3
	2.62	.636	24.2
Av.			24.8
Soft Cooked	2.24	.817	36.4
	2.06	.805	39.0
Av.			37.7

B. Metabolism Experiments.

An analysis of Table 2 shows a remarkable agreement in the percentage of the different samples of egg actually digested. Except for the raw egg period, the percentage digestibility was calculated after subtracting the amount of nitrogen excreted in the feces during a protein-free period. This procedure does away with the complicating factor of endogenous nitrogen. It was impossible to use this method of calculation for the raw egg period because there was less fecal nitrogen excreted during this period than there was during the non-protein period.

This occurrence is difficult to explain although Sherman (17) in reviewing the work of Prausnitz on human beings states that on a liberal diet consisting entirely of non-

nitrogenous food, the amount of nitrogen in the feces was 0.5 to 0.9 gram per day, which is more than is sometimes found in feces from food furnishing enough protein to meet all the needs of the body. Mitchell (16) reports unaccountable variations in the metabolic nitrogen in the feces. It may be that the fluid nature of the raw eggs was the cause, since there was an increase in the fecal nitrogen during the cooked egg periods.

Assuming the small amount of nitrogen in the feces during the raw egg period to be all metabolic nitrogen, there would be no nitrogen from undigested food and the coefficient of digestibility would be 100. This latter figure more likely represents what really happened during Period 2. than the average of 92.2%

which appears in the table.

The hard boiled, scrambled, and soft cooked egg rations resulted in practically the same coefficient of digestibility, the latter being more nearly 100%.

The biological values given in the last column, table 2, were calculated after the method of Mitchell (18). The amount of fecal nitrogen actually derived from the food is obtained by subtracting the fecal nitrogen excreted during a non-protein period from that of the protein period. This figure, subtracted from the total nitrogen ingested gives the amount of absorbed nitrogen. To find how much of the absorbed nitrogen is wasted in metabolism, the amount of urinary nitrogen (corrected in the same manner for the nitrogen of the

catabolism of the body's own tissues) is subtracted from the nitrogen actually absorbed. This figure divided by the absorbed nitrogen times 100 is the biological value of the protein. The calculation resolves itself into the following formula:

$$\frac{N_D - (F_2 - F_1) - (U_2 - U_1)}{N_D - (F_2 - F_1)} \times 100 = \text{Biological Value}$$

where, -

F_1 = Fecal N. during non-protein period

F_2 = Fecal N. during protein period

U_1 = Urinary N. during non-protein period

U_2 = Urinary N. during protein period

N_D = Nitrogen in diet

The biological values show a wide range among rats even upon the same diet. The differences in biological value of the eggs among the animals were as follows: for the raw eggs 33.8, the hard boiled 18.9, the scrambled 15.7, and the soft cooked 5.8. The metabolism data for one rat (427 C) on the last period was omitted because of marked intestinal disturbance. With the exception of the raw egg period, the variations in biological values between several animals is no greater than that obtained by Mitchell (15) for foods at a 10% protein level of intake. With milk protein he found a difference of 18, with corn protein a difference of 23, with oats one of 19, and with potato one of 10. In the experiments reported here, there is as great a variation in biological

value of eggs between animals on the same diet as there is between the values obtained for the same animals on different egg diets. This would seem to indicate that the biological value of eggs is not greatly influenced by the method of cooking.

TABLE 2.

METABOLISM DATA.

Rat No.	Initial Weight	Final Weight	Daily Nitrogen Intake	Daily Fecal Nitrogen	Daily Urinary Nitrogen	Food Nitrogen in Feces	Absorbed Nitrogen	Food Nitrogen in Urine	Total Food Nitrogen Retained	% Digestibility	Biological Value
				Period 1. Protein-free Ration							
427C	249	251	0	.024	.067						
420C	314	316	0	.026	.098						
420B	272	270	0	.028	.086						
427M	259	252	0	.023	.075						
Av.											
				Period 2. Raw Egg Ration							
427C	256	260	.1716	.013	.136	0	.1586	.069	.0896	°92.4	59.7
420C	302	315	.1716	.014	.109	0	.1576	.011	.1466	°91.8	93.5
420B	279	284	.1716	.018	.123	0	.1536	.037	.1116	°89.5	78.4
427M	268	277	.1716	.008	.123	0	.1636	.048	.1156	°95.3	72.0
Av.										°92.2	75.6
				Period 3. Hard Boiled Egg Ration.							
427C	267	264	.1840	.027	.142	.003	.1810	.075	.1060	98.3	56.3
420C	317	315	.1840	.031	.152	.005	.1790	.054	.1250	97.2	69.8
420B	285	287	.1840	.030	.131	.002	.1820	.045	.1370	98.9	75.2
427M	284	279	.1840	.028	.129	.005	.1790	.054	.1250	97.2	69.8
Av.										97.7	67.7
				Period 4. Scrambled Egg Ration							
427C	275	275	.1789	.030	.149	.006	.1729	.082	.0909	96.6	54.0
420C	318	321	.1789	.029	.152	.003	.1759	.054	.1219	98.2	69.7
420B	291	293	.1789	.027	.163	.000	.1789	.077	.1019	100.0	56.9
427M	293	299	.1789	.029	.148	.006	.1729	.073	.0999	96.6	59.1
Av.										97.8	59.9
				Period 5. Soft-cooked Egg Ration							
427C	265	275	.1756	*							
420C	312	318	.1756	.029	.168	.003	.1726	.070	.1026	98.2	59.4
420B	279	292	.1756	.029	.167	.001	.1746	.081	.0936	99.4	53.6
427M	286	298	.1756	.027	.153	.004	.1716	.078	.0936	97.7	54.5
Av.										98.4	55.8

*Intestinal Disturbance

° Uncorrected for Metabolic fecal nitrogen

S U M M A R Y

1. Artificial digestion experiments on eggs prepared in different ways seem to show a slight variation in the rate of digestion. In descending order of speed of digestibility, they are as follows, - soft cooked, hard boiled, raw, and scrambled.

2. Metabolism experiments on rats show no difference in the coefficient of digestibility for the methods of preparation tested.

3. Calculation of the biological values indicates no very marked differenee in the ultimate assimilation and use of the different egg diets.

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