though glycine is a stronger acid than base;[1] with ethyl butyrate the difference in the two solutions is the same in sense but not so great.

Summary.

Glycine, glutamic acid and aspartic acid exert a varying lipolytic action on methyl, ethyl, glyceryl tri- and phenylacetates, ethyl butyrate, and ethyl and phenyl benzoates. If these be arranged in the order of decreasing amounts of hydrolysis, the order will be different in the three cases where the action is caused by water, by glycine, and by glutamic or aspartic acids. This indicates selective action.

The effect of sodium chloride, sodium sulfate, and magnesium sulfate in solutions from 0.2 to 2 normal is not marked or consistent enough to be important for this work.

The hydrolytic action of solutions of glycine and acetic acid on methyl acetate and ethyl butyrate is less than that of corresponding solutions of acetic acid alone; this difference is proportionately much less with ethyl butyrate.

HARRIMAN RESEARCH LABORATORY,
ROOSEVELT HOSPITAL, NEW YORK.

[FROM THE BIO-CHEMICAL LABORATORY OF THE STATION FOR EXPERIMENTAL EVOLUTION, THE CARNEGIE INSTITUTION OF WASHINGTON.]

STUDIES ON THE CHEMISTRY OF EMBRYONIC GROWTH. I. CERTAIN CHANGES IN THE NITROGEN RATIOS OF DEVELOPING TROUT EGGS.[2]

BY ROSS AIKEN GORTNER.

Received March 18, 1913.

Introduction.

The Problem.—The development of an apparently lifeless body into a living, active organism is the one great wonder of the world, and any insight into the chemical processes by means of which this development takes place, must necessarily be of considerable interest. The egg is a chemical laboratory where we find fats, lecithins, cholesterol, carbohydrates, various proteins, a mixture of enzymes, inorganic salts and other compounds. During the development of this egg into an independent animal, all of these compounds are called upon to play their part in the formation of the new structure, but, *do they enter the growing tissue in the same form in which they are laid down in the egg or are synthetic changes also taking place so that some of the material that is present in the egg is used, not in its original form, but in a modified condition?* It seemed to me that synthetic action must take place, for otherwise we must think of the

[1] Lundén, *Sammlung Chem. und Chem.-tech. Vortr.*, 14, 82 (1909).

[2] Read before the Division of Biological Chemistry at the Spring Meeting of the American Chemical Society, Minneapolis, Minn., March, 1913.

(probably) comparatively simple egg proteins, ovalbumen, ovomucoid, ovoglobulin, etc., as containing, not only all of the amino acids necessary for the formation of the (probably) more complex proteins such as the keratins or hemoglobin, but also *that each amino acid is present in the egg in exactly the quantity that will be later needed by the growing organism.*

Of the many elements which enter into the composition of the egg there is one, nitrogen, that we can determin with great accuracy, and we can also determin some of the different groups and compounds which contain this nitrogen by making use of certain characteristic reactions. Nitrogen is also probably one of the most interesting of the cell components, for through a study of the nitrogen fractions we gain an insight into the problem of the protein structure, and the utilization of the egg proteins by the growing embyro. This paper is, therefore, devoted to a study of the forms of nitrogen that occur in the fresh eggs and in eggs at various stages of development.

The Material.—Suitable material in abundance is hard to procure. Hen eggs are so large that if there is any individual difference, as there is almost certain to be unless the eggs all come from chickens of the same race, a very large composit sample would be necessary to eliminate all possible errors due to sampling. Most of the fish eggs are small and cannot be accurately counted without excessive labor.[1] Suitable material was found in the eggs of the brook trout (*Savelinus fontinalis L.*). These eggs are about 3–4 mm. in diameter and weigh in the neighborhood of 20 milligrams when dry. The egg contains about 11.5% of nitrogen. My original sample of several thousand eggs came from a large lot of over a million eggs, and from the smaller sample I took from 500 to 1000 eggs at intervals of 10–20 days, beginning with eggs less than 24 hours' old and ending with young fish, whose yolk sac was almost completely absorbed, but who had never received food. The possibility of food being taken from the water in which the eggs were hatched is precluded by the fact that the water comes from artesian wells and contains no animal life.

The material was kindly given me by Mr. Charles Walters, in charge of the Cold Spring Harbor Hatchery of the N. Y. State Fish Commission, and I wish to take this opportunity of expressing my gratitude.

Historical.—Considerable work has already been done on the chemistry of developing embryos. Tichomiroff (1885) in a study of the developing eggs of the silk worm (*Bombyx mori*) concluded that the eggs lost protein, glycogen, fat and cholesterol, and gained lecithins and purine bases.

[1] Weighing out the same quantity of eggs could not be practised because changes in weight occur during development. If the same number of eggs is taken, and the number be sufficiently large to average individual differences in size, the changes due to growth can be easily followed.

Mendel and Leavenworth (1908) later showed that the loss of cholesterol and the gain of purines also held for developing hen and duck eggs.

Levene (1902) has shown that the proteins must be broken down during embryonic growth, for he isolated free amino acids from embryonic tissue. Abderhalden and Kempe (1907) determined the tyrosine, glycocoll and glutamic acid in fresh and developing hen eggs and concluded that the formation of an embryo did not alter the quantity of these amino-acids, although somewhat more tyrosine was isolated from the chicks at hatching than was found in the fresh eggs.

Tangl and his co-workers (1903–'09) have attacked the problem from a somewhat different point. They have made certain chemical analyses and in addition have determined the loss of energy (Entwicklungsarbeit) by means of a calorimeter. They find in all of the varieties of eggs which they have examined that during development, carbon dioxide, and water are lost but that no nitrogen leaves the egg until after the egg membrane is broken. In the hen egg the development apparently takes place solely at the expense of the fat, which is "burned" to carbon dioxide and water, to furnish the "Entwicklungsarbeit." In silk worm eggs they find (Farkas 1906) that approximately two-thirds of the energy comes from the fats and the other third from either the proteins or the carbohydrates.

Of more importance for our purpose, however, is the study made by Tangl and Farkas (1904) of the energy loss and the chemistry of the development of the eggs of the European trout. The results of their analyses are of such importance that I have appended a summary in the table below. The figures are based upon 518 eggs in each instance.

		Fresh eggs.	Hatched eggs.	Loss.
	Weight	45.70 gr.	43.16 gr.	—2.54 gr.
	Dry substance	15.49 gr.	15.08 gr.	—0.41 gr.
Fat	"nach Lieberman's verseifungs Methode"	3.31 gr.	3.44 gr.	+0.13 gr.
Fat	Ether extract	1.46 gr.	1.84 gr.	+0.38 gr.
	Nitrogen	1.86 gr.	1.85 gr.	—0.01 gr.
	Carbon	8.67 gr.	8.48 gr.	—0.19 gr.
	Energy	99.58 kg. cal.	96.39 kg. cal.	—3.46 kg. cal.

It will be noted that there is only a slight loss of nitrogen, not greater they believe, than the experimental error, and that there is a *gain* of fats. Carbon dioxide is constantly eliminated. Tangl and Farkas explain these findings by assuming that the "Entwicklungsarbeit" comes, not from the fat as is the case in hen eggs, but from the protein, inasmuch as glycogen is absent in the fresh eggs. They reason that only the glyco-proteins can form fats, so that in these complex proteins must be found the source of the energy of development. Each egg expends 6.68 small calories in the process of developing to the hatching stage, and Tangl and Farkas theorize that to produce this energy in 518 eggs, 1.67 grams of glyco-

protein (9.7 Cal.) must be broken down to 0.38 gram of fat (3.5 Cal.) 0.30 gram of glycogen (1.3 Cal.) and all of the nitrogen retained in the form of urea (or uric acid) (urea = 0.57 gram = 1.40 Cal.), the difference between these heat values being carbon dioxide and water with a heat value of 3.5 Cal. while their experiments showed a loss of 3.46 Cal. They add that the loss of carbon (46.3%) does not agree with the expected loss (68%), and suggest that perhaps all of the carbon is not eliminated as carbon dioxide, but is retained in the organism in some other form. Later in this paper I shall have some comment to make on this theorizing.

Experimental.

The Method.—Each sample consisted of 400 trout eggs, with the exception of Sample 4 which contained the fish from 467 eggs. This variation was necessitated because the fish were dried before counting and it was found that they could not be separated without loss of material. The calculations on this sample are corrected to correspond with the other lots. The eggs were spread out as soon as procured, in a single layer on the cover of a Petri dish and dried in an oven heated by boiling water. The dry weight was then ascertained. The entire sample was carefully removed to a special 500 cc. Erlenmeyer flask, fitted with an inverted Hopkins condenser by means of a ground glass joint, 100 cc. of hydrochloric acid of 1.115 sp. gr. was added, and the mixture hydrolyzed for exactly 48 hours by boiling gently on an electric hot plate. The outlet from the condenser was so arranged that all fumes entering the apparatus must pass over dilute hydrochloric acid in a wash bottle, thus preventing all possibility of the absorption of ammonia from the air of the laboratory.

After the completion of the 48-hour hydrolysis the mixture was evaporated in a distilling flask under diminished pressure until all of the hydrochloric acid possible had been driven off, and then the residue was analyzed according to Van Slyke's method (1911) for the determination of the chemical groups characteristic of the various amino acids. Some minor changes will be noted in the method, notably as regards certain dilutions. These changes were necessitated by that fact that the material hydrolyzed did not consist of a pure protein, but contained fats, etc., *each lot, however, was subjected to exactly the same method of analysis so that the results are strictly comparable.* It may be objected that other substances which contain nitrogen are present besides proteins, and that this would vitiate the results. It is, no doubt, true that there are other substances present, for example, lecithins, purine bases, etc., but these substances are also present in the egg and inasmuch as all samples were treated exactly alike, the results must be comparable, and that is all that I claim. In my table I list the results under the fractions "arginine," histidine," etc., but I do not claim that these fractions are, necessarily, pure arginine or histidine but merely that *they contain those nitrogen fractions which correspond*

in their chemical characteristics to the pure arginine, histidine, etc., of the proteins.

Certain changes are doubtless brought about by the acid hydrolysis. The nitrogen of the lecithins is split off as choline, and probably a considerable portion if not all of the choline nitrogen is obtained in the "ammonia" fraction. Some of the purines are doubtless broken down to simpler compounds, but here again the same reaction takes place in each fraction so that the *comparative* results are not altered.

The residue, after distilling off the excess of hydrochloric acid, was dissolved in 100–150 cc. of water, 100 cc. of 95% ethyl alcohol and an excess of a 10% suspension of calcium hydroxide was added and the ammonia distilled off into standard acid at a temperature of 40–45° under a pressure of less than 30 mm. The results are listed under *Ammonia N.*

The alkaline mixture in the distilling flask was filtered and the precipitate, containing the humin, most of the fats, etc., was well washed with hot water. A Kjeldahl determination was then made of the filter and its contents, and the nitrogen found listed under *humin.*

The filtrate and washings from the humin were acidified and evaporated under diminished pressure to less than 200 cc. and after cooling made to 200 cc. volume. Duplicate Kjeldahl determinations were made on 10 cc. portions of this solution and the results are listed under *total nitrogen in the filtrate from the humin.*

The *total nitrogen in the egg* was determined by adding the nitrogen obtained as ammonia and humin to the total nitrogen in the filtrate from the humin. This modification of Van Slyke's method was necessitated by the fact that the presence of fats caused the humin to ball together, so that an accurate aliquot portion could not be obtained before the humin and fats were removed.

To 160 cc. of the filtrate from the humin I added 18 cc. of concentrated hydrochloric acid and heated on a water bath until hot. I then added a solution containing 25 grams of purified phosphotungstic acid[1] and continued the warming for an hour or more. The flask was then set aside in a cool place for 48 hours. The precipitate was then filtered off and washed as directed by Van Slyke.

The basic phosphotungstates were suspended in 800 cc. of water and brought into solution by the cautious addition of sodium hydroxide solution, a few drops of phenolphthalein being added to guard against too great an excess of the alkali. The phosphotungstic acid was then precipitated with a slight excess of 20% barium chloride, and the barium phosphotungstate well washed with hot water. The filtrate and washings were united, and evaporated under diminished pressure to a small volume,

[1] Van Slyke recommends 15 grams, but I found that with this material complete precipitation did not take place when only 15 grams were used.

filtered and made to 50 cc. volume. *Arginine* and *total nitrogen in the bases* were determined in 25 cc. of this solution as directed by Van Slyke. *Cystine* was determined in the solution of the bases by obtaining the sulfur content of a 10 cc. portion and *amino nitrogen in the bases* was determined on duplicate portions of 5 cc. each by means of Van Slyke's apparatus (1911, 1912.)

The filtrate from the bases was almost neutralized by the addition of sodium hydroxide solution, and then concentrated under diminished pressure to crystallization. On cooling the residue was made to 200 cc. volume. *Total nitrogen in the filtrate from the bases* was determined by Kjeldahl on 25 cc. portions and the *amino nitrogen in the filtrate from the bases* was determined in Van Slyke's apparatus, using 10 cc. portions of the solution.

The analytical data follow:[1]

Sample 1.—400 trout eggs, stripped and fertilized on Oct. 30, 1912. Secured and drying begun at 9 A.M. Oct. 31st.

Weight of 400 eggs, moist, 25.24 grams.

Weight, dry at 100°, 7.5404 grams.

Dry materials in eggs, 29.87 per cent.

Ammonia N = 47.20 cc. 0.1 *N* acid, indicating 0.0661 gram of ammonia N in 400 eggs.

Humin N = 11.38 cc. 0.1 *N* acid indicating 0.0159 gram of humin N in 400 eggs.

Nitrogen in the filtrate from the humin = 28.08 and 28.46 cc. 0.1 *N* acid the average, indicating 0.7915 gram of nitrogen in the 400 eggs.

Total nitrogen = ammonia N + humin N + N in the filtrate from the humin = 0.8735 gram N in the 400 eggs.

Arginine N = 14.25 cc. 0.1 *N* acid = 0.0999 gram N or 0.0968 gram when corrected for cystine.[2]

Total nitrogen in the bases = 71.25 cc. 0.1 *N* acid (including the arginine N), indicating 0.2498 gram of basic nitrogen in the 400 eggs.

Cystine = 0.0079 gram $BaSO_4$ indicating 0.0030 gram of cystine N in the 400 eggs.

Amino nitrogen in the bases = (1) 19.00 cc. N at 20° and 763 mm. (2) 19.10 cc. N at 20° and 761 mm., the average indicating 0.1360 gram of amino N in the 400 eggs.

Histidine nitrogen, calculated according to Van Slyke = 0.0772 gram N in the 400 eggs.

Lysine nitrogen calculated according to Van Slyke = 0.0728 gram N.

Total nitrogen in the filtrate from the bases = 38.35 and 38.45 cc. 0.1 *N* acid, indicating an average of 0.5376 gram N.

Amino nitrogen in the filtrate from the bases = (1) 35.20 cc. N and (2) 35.40 cc. N at 20° and 761 mm., indicating 0.5036 gram of amino N in the filtrate from the bases.

These data, calculated to *per cents. of the total nitrogen*, form the first column in Table I.

Sample 2.—400 trout eggs secured 9 A.M. Nov. 21st. Eyes easily visible, red blood present. Move in membrane.

[1] All calculations were made with the aid of a four-place table of logarithms.

[2] See Van Slyke, *Loc. cit.*

Weight of 400 eggs, moist, = 23.85 grams.

Weight, dry, = 7.4920 grams.

Dry materials in the egg = 31.42%.

Ammonia N = 56.0 cc. 0.1 *N* acid, indicating 0.0784 gram of N.

Humin N = 14.35 cc. 0.1 *N* acid, indicating 0.0201 gram of N.

Nitrogen in the filtrate from the humin = 28.25 and 28.35 cc. 0.1 *N* acid, indicating an average of 0.7923 gram N.

Total nitrogen = 0.0784 + 0.0201 + 0.7923 = 0.8908 gram N in 400 eggs.

Arginine N = 15.25 cc. 0.1 *N* acid, indicating 0.1068 gram N or 0.1033 gram when corrected for cystine.

Total N in the bases = 74.70 cc. 0.1 *N* acid (including the arginine N), indicating 0.2614 gram of N.

Cystine N = 0.0094 gram $BaSO_4$, indicating 0.0035 gram N.

Amino N in the bases = 20.0 and 20.0 cc. N at 20° and 761 mm., indicating 0.1424 gram of amino N.

Histidine N (calculated) = 0.0783 gram N.

Lysine N (calculated) = 0.0763 gram N.

Total N in the filtrate from the bases = 37.55 and 37.55 cc. 0.1 *N* acid, indicating 0.5257 gram of N.

Amino N in the filtrate from the bases = 34.10 cc. N at 19° and 761 mm. and 34.20 cc. N at 20° and 761 mm., the average indicating 0.4876 gram of N.

These data, calculated to *per cents of the total nitrogen*, form the second column in Table I; and the *difference between these data and those of the fresh eggs, in gram*, form the first column in Table II.

Sample 3.—400 trout eggs secured 11 A.M. Dec. 3rd. Eyes deeply pigmented, body twice as long as the diameter of the egg. The heart is seen to beat and there is a network of veins and arteries. Will hatch in about two weeks.

Weight of 400 eggs, moist, = 24.47 grams.

Weight, dry at 100°, 7.5287 grams.

Dry materials in the egg, 30.77%.

Ammonia N = 48.30 cc. 0.1 *N* acid, indicating 0.0676 gram N.

Humin N = 14.25 cc. 0.1 *N* acid, indicating 0.0200 gram N.

Nitrogen in the filtrate from the humin = 27.50 and 27.70 cc. 0.1 *N* acid, indicating 0.7727 gram of N.

Total N = 0.0676 + 0.0200 + 0.7727 = 0.8603 gram N in 400 eggs.

Arginine N = 17.05 cc. 0.1 *N* acid, indicating 0.1195 gram N or 0.1147 gram when corrected for cystine.

Total N in the bases = 80.20 cc. 0.1 *N* acid (including Arg. N), indicating 0.2807 gram of basic N.

Cystine N = 0.0120 gram $BaSO_4$, indicating 0.0045 gram of N.

Amino N in the bases = 22.00 and 22.10 cc. N at 19° and 763 mm., the average indicating 0.1585 gram of N.

Histidine N (calc.) = 0.0692 gram N.

Lysine N (calc.) = 0.0917 gram N.

Total N in the filtrate from the bases = 34.95 and 34.95 cc. 0.1 *N* acid, indicating 0.4893 gram N.

Amino N in the filtrate from the bases = 31.40 cc. N at 21° and 774 mm. and 31.30 cc. N at 20° and 774 mm., indicating 0.4541 gram N.

These data, calculated to *per cents of the total nitrogen*, form the third column in Table I, and the *difference between these data and those of the fresh eggs, in gram*, forms the second column in Table II.

Sample 4.—467 trout eggs, just hatched, yolk sac very large.

Weight, dry at 100°, = 8.2505 grams.

Weight of 400 fish (calc.), 7.0668 grams.

Ammonia N = 60.30 cc. 0.1 *N* acid, indicating 0.0723 gram N in 400 eggs.

Humin N = 18.55 cc. 0.1 *N* acid, indicating 0.0222 gram N in 400 eggs.

Nitrogen in the filtrate from the humin = 30.45 and 30.35 cc. 0.1 *N* acid, indicating 0.7291 gram N in 400 eggs.

Total N = 0.0723 + 0.0222 + 0.7293 = 0.8238 gram N in 400 eggs.

Arginine N = 17.40 cc. 0.1 *N* acid, indicating 0.1043 gram N or 0.1009 gram in 400 eggs when corrected for cystine.

Total N in the bases = 81.80 cc. 0.1 *N* acid (incl. Arg.), indicating 0.2452 gram N in 400 eggs.

Cystine N = 0.0103 gram $BaSO_4$, indicating 0.0033 gram N in 400 eggs.

Amino N in the bases = 23.00 cc. N at 20° and 755 mm., and 23.10 cc. N at 20° and 754 mm., indicating 0.1394 gram N in 400 eggs.

Histidine N (calc.) = 0.0758 gram N in 400 eggs.

Lysine N (calc.) = 0.0651 gram N in 400 eggs.

Total N in the filtrate from the bases = 38.45 and 38.25 cc. 0.1 *N* acid, indicating 0.4599 gram N in 400 eggs.

Amino N in the filtrate from the bases = 35.00 cc. N at 22° and 769 mm., and 35.40 cc. N at 23° and 767 mm., indicating 0.4284 gram N in 400 eggs.

These data, calculated to *per cents of the total nitrogen*, form the fourth column in Table I, and the *difference between these data and those of the fresh eggs, in gram*, forms the third column in Table II.

Sample 5.—400 young trout. Secured Jan. 9, 1913. Yolk sac very small. Are just about ready to take food.

Weight of 400 fish, dry at 100°, = 5.6295 grams.

Ammonia N = 44.75 cc. 0.1 *N* acid, indicating 0.0627 gram N.

Humin N = 13.30 cc. 0.1 *N* acid, indicating 0.0186 gram N.

Nitrogen in the filtrate from the humin = 21.45 and 21.45 cc. 0.1 *N* acid, indicating 0.6005 gram N.

Total N = 0.0627 + 0.0186 + 0.6005 = 0.6818 gram N.

Arginine N = 12.70 cc. 0.1 *N* acid, indicating 0.0891 gram N or 0.0854 corrected for cystine.

Total N in the bases = 66.35 cc. 0.1 *N* acid (incl. Arg.) indicating 0.2322 gram N.

Cystine N = 0.0092 gram $BaSO_4$, indicating 0.0035 gram N.

Amino N in the bases = 17.30 and 17.10 cc. N at 19.5° and 761 mm., indicating 0.1230 gram N.

Histidine N (calc.) = 0.0819 gram N.

Lysine N (calc.) = 0.0615 gram N.

Total N in the filtrate from the bases = 26.40 and 26.45 cc. 0.1 *N* acid, indicating 0.3705 gram N.

Amino N in the filtrate from the bases = 24.00 cc. N at 19° and 762 mm., and 24.20 cc. N at 21° and 762 mm., indicating 0.3439 gram N.

These data, calculated to *per cents of the total nitrogen*, form the fifth column in Table I, the *difference between these data and those of the fresh eggs, in gram*, forms the fourth column in Table II, and the *various fractions of nitrogen lost, calculated to per cents of the total nitrogen lost*, as compared with the fresh eggs, are given in Table III.

TABLE I.—THE DISTRIBUTION OF THE NITROGEN IN PER CENTS. OF THE TOTAL NITROGEN.

Age of trout eggs:	1 day.	21 days.	35 days.	51 days.	72 days.
Ammonia N	7.56	8.80	7.86	8.78	9.19
Humin N	1.82	2.26	2.32	2.70	2.73
Arginine N	11.09	11.60	13.32	12.26	12.52
Cystine N	0.34	0.40	0.52	0.40	0.50
Histidine N	8.83	8.79	8.04	9.20	12.01
Lysine N	8.33	8.57	10.66	7.91	9.02
Non-amino N in the filtrate from the bases	3.90	4.27	4.09	3.85	3.90
Amino N in the filtrate from the bases	57.65	54.73	52.78	52.00	50.43
Total	99.52	99.42	99.59	97.10	100.30
Total basic N	28.60	29.34	32.63	29.78	34.06
Mon-amino acid N	61.55	59.00	56.87	55.85	54.33

TABLE II.—THE GAIN OR LOSS OF THE VARIOUS FORMS OF NITROGEN IN GRAMS, DURING THE DEVELOPMENT OF 400 EGGS.

Age of trout eggs:	21 days.	35 days.	51 days.	72 days
Total basic N	+0.0106	+0.0309	—0.0046	—0 0176
Non-amino N in filtrate from bases	+0.0041	+0.0012	—0.0025	—0.0074
Amino N in filtrate from bases	—0.0160	—0.0495	—0.0752	—0.1597
Ammonia N	+0.0124	+0.0015	+0.0062	—0 0034
Humin N	+0.0042	+0.0041	+0.0063	+0.0027
Arginine N	+0.0065	+0.0227	+0.0041	—0.0114
Cystine N	+0.0006	+0.0015	+0.0003	+0.0005
Histidine N	+0.0011	—0.0080	—0.0014	+0.0047
Lysine N	+0.0035	+0.0189	—0.0077	—0.0113
Total loss or gain of N as compared with Sample 1	+0.0172	—0.0133	—0.0500	—0.1918

TABLE III.—THE VARIOUS FRACTIONS OF THE NITROGEN LOST, CALCULATED TO PER CENTS. OF THE NITROGEN LOST, IN THE PERIOD OF DEVELOPMENT FROM THE EGG TO A COMPLETE FISH (1 DAY TO 72 DAYS).[1]

	Gram.	Per cent.
Total nitrogen lost	0.1918[2]	....
Ammonia N lost	0.0034	1.77
Arginine N lost	0.0114	5.94
Lysine N lost	0.0113	5.89
Amino N in the filtrate from the bases	0.1597	83.30
Non-amino N in the filtrate from the bases	0.0074	3.86
Total		100.76
Total basic N lost	0.0176	9.17
Mon-amino acid N lost	0.1671	87.14

[1] The humin, histidine and cystine are omitted from the figures given below, because slight *gains* are recorded in their content (see Table II). It seems probable that these gains are not more than the experimental error, and that there is neither loss nor gain of these fractions. There may be a slight gain of humin, due to the melanin formed.

[2] This number is not necessarily the sum of the numbers below (0.1932 gram), inasmuch as this figure was obtained by subtracting the total N of Sample 5 from the

Colorimetric Estimation of Phenols.—Folin and Denis(1912 (*b*)) have recently published a new colorimetric method for the determination of tyrosine[1] and it seemed worth the while to determin the phenolic content of the "filtrate from the bases," the fraction in which tyrosine would occur.

Portions of 2 cc. each of the filtrate from the bases of Samples 1, 3 and 5, were treated with 5 cc. of Folin's phenol reagent (Folin and Denis, 1912 (*a*)) and the mixture allowed to stand for 5–10 minutes. Then 25 cc. of a saturated solution of sodium carbonate was added, the whole diluted to 100 cc., and after standing for 10 minutes for the color to completely develop, the depth of color was read against a standard color, produced by a solution of known tyrosine content.

Phenols equivalent to 0.2500 gram of tyrosine were present in the 400 eggs of Samples 1 and 3, and this diminishes to 0.1775 gram on Sample 5, showing a loss of 29% of the phenolic compounds originally present. The depth of color in Samples 1 and 3 was apparently identical.

Discussion.

The Loss of Total Nitrogen.—By referring to Table II, it will be seen that probably no nitrogen was either lost or gained until after hatching. In Sample 2 there is a gain of 0.0172 gram N in the total nitrogen content of the 400 eggs, and in Sample 3 there is a corresponding loss of 0.0133 gram. It seems probable that I did not use a large enough composit sample to avoid a slight variation in the total nitrogen content. Then again, an error of ± 0.5 cc. of 0.1 *N* acid in the determination of the total nitrogen in the filtrate from the humin would cause a $\pm$ error of 0.0140 gram in the total nitrogen.

After the fish are hatched there is a rapid loss of nitrogen. Very probably a considerable portion of the nitrogen lost in Sample 4 is due to the loss of the keratin-like egg membrane, which, under the conditions at my disposal, could not be secured for analysis. Following hatching the loss proceeds rapidly until 21 days after hatching the fish have lost 25.35% of their dry weight and 21.96% of their total nitrogen, showing

total N of Sample 1, while the numbers for the various fractions were obtained by subtracting the fractions of Sample 5 from similar fractions of Sample 1. The close agreement found (100.76%) speaks well for the accuracy of the analyses.

[1] Folin and Denis believe that tyrosine is responsible for the total color produced when pure proteins are hydrolyzed. I have already shown (Gortner, 1911) that in some proteins it is possible to obtain a strong Millon's reaction after the tyrosin has been removed by crystallization and after a second lot of crystals (probably impure leucine) have been removed *which do not give Millon's reaction.* The substance giving the reaction can be concentrated in the most insoluble fraction (tyrosine), *and in the most soluble fraction,* intermediate fractions giving no reaction. It therefore seems probable to me that some other phenolic substance besides tyrosine is present in at least some of the proteins, or that it is produced under the usual conditions of hydrolysis.

that the protein (N × 6.25) is utilized to furnish energy to a greater extent than are the non-nitrogenous bodies.

Changes in the Form of Nitrogen during Development.—If the analyses of Samples 1 and 2 were the only analyses available I should say that they were near enough alike to differ by not much more than the experimental error. When, however, we compare all of the data, each column with that preceding and following, we note that there is a constant trend in one direction, *from the non-basic nitrogen toward the basic nitrogen.* Perhaps this means only what Tichomiroff (1885) and Mendel (1908) have already shown, that purines are synthesized, but in this instance I have shown that the compounds from which they are formed are, in all probability, the monamino acids. It seems possible, with the almost infinite multiplication of cells that the purines needed to form the nucleic acids must be synthesized from less complex bodies.

I have already noted that Tangl and Farkas (1904) explain their calorimetric findings by supposing that the energy of development (to hatching) comes from the breaking down of glycoproteins and that all of the nitrogen is "retained in the form of urea (or uric acid)." By a glance at Table II it can be easily seen that no great amount of urea can be present at any one time. Tangl and Farkas' hypothesis would necessitate the presence of 0.44 gram of urea in the fish at the time of hatching. The nitrogen in the urea under the conditions of my experiments would appear as ammonia N necessitating a change of +0.2053 gram of ammonia N. At 35 days' development the ammonia N had increased not more than three times the experimental error (+0.0015 gram). Even at hatching if all of the nitrogen lost (0.0500 gram) were counted as urea nitrogen and added to the increased ammonia N we would still have only a little more than one-fourth enough urea N to agree with their hypothesis.

I could find no data as to the effect of boiling uric acid with hydrochloric acid at ordinary pressure, so I instituted the following experiment: 0.1434 gram uric acid (weight calculated from the total N content) was boiled under the same conditions as the hydrolyzed eggs, for 48 hours with 25 cc. HCl, sp. gr. 1.115. The ammonia N, humin N (no dark color was produced, the N obtained here was probably that of calcium ureate), basic N, and non-basic N, were determined with the following results:

Ammonia N	5.20 cc. 0.1 *N* acid	0.0073 gram N	15.27%
Humin N	12.30 cc. 0.1 *N* acid	0.0172 gram N	35.98%
Basic N	4.40 cc. 0.1 *N* acid	0.0062 gram N	12.97%
Non-basic N	12.20 cc. 0.1 *N* acid	0.0171 gram N	35.78%

If Tangl and Farkas' second hypothesis be true, *i. e.*, that the nitrogen of the glycoprotein is retained as uric acid, we should have a change of +0.0738 gram in our humin fraction and +0.0313 gram in our ammonia N, neither of which is anywhere near approached, especially the humin

N. We must therefore look for some explanation other than that given by Tangl and Farkas.

Perhaps the change from non-basic nitrogen to basic nitrogen is accompanied by a loss of heat, but I have no means at my disposal to test this hypothesis. My tables show that *the various forms of nitrogen are not fixed quantities*, but that the ratios shift as development progresses, and in this shifting it seems very reasonable to suppose that energy relations are changed, and that some energy may be liberated as heat.

It also seems very probable, in view of my results from the uric acid hydrolysis, that my gain of basic nitrogen would have been much greater under milder conditions of hydrolysis, inasmuch as only 13% of the uric acid nitrogen was obtained as basic nitrogen.

The Composition of the Nitrogen Lost during the 72 Days' Development.—When the young fish ready to take food were taken for analysis, it was found that they had lost 21.90% of the total nitrogen contained in the eggs. The composition of this lost nitrogen may be seen in Table III, where both grams and per cent. loss are given. This table is especially significant inasmuch as it shows that there is a selective utilization of the various nitrogen fractions, and that a greater percentage of certain nitrogenous compounds is retained in the tissues of the new organism. Were there no selective elimination, the nitrogen lost would contain the various fractions in the same percentage as in Sample 1, Table I. We note, however, that only approximately 25% of the expected quantity of ammonia N is eliminated, only approximately 50% of the expected arginine reacting nitrogen is eliminated, only approximately 75% of the expected lysine reacting nitrogen, none of the histidine reacting nitrogen (there is a slight gain), the cystine is all retained in the new organism, and of the total basic nitrogen, only about one-third of the expected quantity is eliminated. The mon-amino nitrogen in the filtrate from the bases is used uniformly, while the amino nitrogen in the filtrate from the bases, probably nearly all mon-amino acid nitrogen, is utilized far in excess of the expected proportion.

I believe, therefore, that I am safe in saying that my chemical study shows that while 37.26% of the energy of development, *as indicated by the loss of chemical substances*, including the time from the fertilization of the egg until the fish are ready to take food, comes from the non-proteins, that 62.73% must come from the proteins (loss of N $\times$ 6.25), and that the greater part of the protein contribution comes from the mon-amino acids, most of the basic nitrogen of the egg being retained in the growing organism.

Summary.

In a study of the various fractions of nitrogen in developing trout eggs it was found that:

(1) Probably no nitrogen is lost from the egg up to the time of hatch-

ing. After hatching the loss of nitrogen proceeds rapidly, until at the end of 21 days after hatching 21.96% of the total nitrogen in the egg has been lost.

(2) The eggs lose 25.35% of their dry weight during the development from the egg to the fish, 37.26% of this loss being due to non-proteins (fats, etc.), and 62.73% to proteins.

(3) During the process of development, basic nitrogen increases in the egg at the expense of the mon-amino acid nitrogen.

(4) It has been shown that the hypothesis postulated by Tangl and Farkas to account for the energy of development of the trout egg is incorrect, inasmuch as no considerable quantities of either urea or uric acid are formed during the development of the egg.

(5) There is a selective utilization of the various nitrogen fractions by the developing fish, as is shown by the composition of the nitrogen lost. Only 25% of the expected amide N is eliminated, only 50% of the expected arginine reacting N, only 75% of the expected lysine reacting N, none of the cystine or histidine reacting N is eliminated, only about one-third of the expected basic nitrogen, while the deficit caused by the basic nitrogen is filled by the elimination of mon-amino acid nitrogen far in excess of the expected quantity (83.30% of the total N: expected, 57.65%).

(6) It seems probable that some of the energy of development (Entwicklungsarbeit) comes from the shifting of the nitrogen ratios, as development proceeds. In the change from mon-amino acid nitrogen to basic nitrogen the energy relations may be changed and heat liberated, but at present this is only a hypothesis.

Literature Cited.

Abderhalden and Kempe (1907), "Vergleichende Untersuchungen über den Gehalt von befruchteten Hünereiern in verschiedenen Entwicklungsperioden an Tyrosine, Glykokoll, und an Glutaminsäure," *Z. Physiol. Chem.*, **53**, 398–402.

Folin and Denis (1912), (*a*) "On Phosphotungstic-Phosphomolybdic Compounds as Color Reagents," *J. Biol. Chem.*, **12**, 239–43; (*b*) "Tyrosine in Proteins as Determined by a New Colorimetric Method," *J. Biol. Chem.*, **12**, 245–51.

Gortner (1911), "A new Decomposition Product from Keratin which gives Millon's Reaction" (Preliminary note), *J. Biol. Chem.*, **9**, 355–7.

Levene (1902), "Embryochemische Untersuchungen," *Z. Physiol. Chem.*, **35**, 80–3.

Mendel and Leavenworth (1908), "Chemical Studies on Growth. VI. Changes in the Purine, Pentose and Cholesterol Content of the Developing Egg," *Amer. Jour. Physiol.*, **21**, 77–84.

Tangl, *et al.* (1903–12), "Beiträge zur Energetik der Ontogenese;" (1903), "I. Die Entwicklungsarbeit im Vogelei," *Arch. ges. Physiol.*, **93**, 327–75.

Forkas (1906), "III. Ueber die Energieumsatz des Seidenspinners während der Entwicklung im Ei und während der Metamorphose," *Arch. ges. Physiol.*, **98**, 490–545.

Tangl and Farkas (1904), "IV. Ueber der Stoff-u. Energieumsatz im bebrüteten Forellenei," *Arch. ges. Physiol.*, **104**, 624–38; u. Mituch (1908), "V. Weitere Unter-

suchungen über die Entwicklungsarbeit und den Stoffumsatz im bebrüteten Hünerei," *Arch. ges. Physiol.*, **121**, 437–58; (1909), "VII. Embryonale Entwicklung u. Metamorphose vom energetischen Standpunkte aus betrachtet," *Arch. ges. Physiol.*, **130**, 55–89.

Glaser, "VIII. Die Entwicklungsarbeit bei Fundulusei," *Biochem. Z.*, **44**, 180–4. (See *Science, N. S.*, **35**, 189–91 (1912).)

Tichmiroff (1885), "Chemische Studien über die Entwicklung der Insecteneier," *Z. Physiol. Chem.*, **9**, 518–32.

Van Slyke (1911), (*a*) "The Analysis of Proteins by Determination of the Chemical Groups Characteristic of the Different Amino-acids," *J. Biol. Chem.*, 10, 15–55; (1911), (*b*) "The Quantitative Determination of Aliphatic Amino Groups, *J. Biol. Chem.*, **9**, 185–204; (1912), "The Quantitative Determination of Aliphatic Amino Groups. II," *J. Biol. Chem.*, 12, 275–84.

NEW BOOKS.

The Freezing-Point, Boiling-Point and Conductivity Methods. Second edition, completely revised. By HARRY C. JONES, Professor of Physical Chemistry in the Johns Hopkins University. Easton, Pa.: The Chemical Publishing Co. 1912. 14 × 20 cm., vii + 75 pp. Cloth, $1.00.

In view of the wider range of laboratory manuals of physical chemistry that have become available since 1897, the date of the first edition of this little work, it is probable that this second edition, with a field limited to three methods, will fill a want felt hardly so strongly as formerly. It may be recalled that the author aims to give, not only an account of the mechanical operations involved in carrying out these methods in the laboratory, but also enough of the theoretical ground on which each of them rests to enable the student to work with them intelligently and to see clearly their scientific significance and use. In a volume so small, however, there is not always enough space to deal adequately with the theoretical side; for example, one cannot but feel that, to the uninitiated, the thermodynamic deduction on p. 7 must savor of hocus pocus, especially in the absence of any mention of possible reversibility.

Most of the text remains the same, but the numerical data have been amplified, a roller bridge is figured and described, as also is a newer type of thermostat. Nothing is said of any of the Landsberger types of boiling-point apparatus. ALAN W. C. MENZIES.

Conférences sur Quelques Thèmes Choisis de la Chimie Physique, Pure et Appliquée. By SVANTE ARRHENIUS, Directeur de l'Institut Nobel scientifique, à Stockholm. Paris: Libraire Scientifique, A. Hermann & Fils. 1912. 14 × 23 cm., 113 pp. Paper covers, 3 fr.

This series of five lectures was given by Arrhenius at the University of Paris in March, 1911. The titles are: "La Théorie Moleculaire;" "Les Suspensions et les Phénomènes d'Adsorption;" "L'Energie Libre;" "Les Atmosphères des Planètes" (issued separately, and reviewed in THIS JOURNAL, **34**, 1740 (1912)); and "Les Conditions Physiques sur La Planète Mars."