was present in a more completely hydrolyzed state, in the plant than in the yeast or meat extracts.

No creatine or creatinine and very little purine nitrogen was found in the plant extracts. The yeast extract showed high purine nitrogen and no creatine or creatinine. Plant and yeast extracts gave no biuret reaction but the meat extracts examined gave this reaction.

All of the nitrogen of the plant extracts was found in the filtrate after applying acid-alcohol, and consists chiefly of mono-amino acids and ammonia. About 25% of the nitrogen of the meat and yeast extracts is precipitated by acid-alcohol. The plant extracts yield a much larger percentage of nitrogen as ammonia nitrogen than the meat or yeast extracts.

In differentiating these three classes of extracts, the creatinine, ammonia, purine nitrogen and acid-alcohol determinations are of value to the food analyst.

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STUDIES ON THE CHEMISTRY OF EMBRYONIC GROWTH. II. COMPARATIVE ANALYSES OF THE EGGS AND OF THE NEWLY-HATCHED LARVAE OF THE GIANT SALAMANDER, CRYPTOBRANCHUS ALLEGHENIENSIS.

BY ROSS AIKEN GORTNER.

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Introduction.

The Problem.—The purpose of this investigation is identical with that of the first paper in this series (Gortner, 1913), *i. e.*, to determine whether the "chemical compounds which are present in the egg enter the growing tissues in the same form in which they are laid down in the egg, or whether synthetic changes are also taking place so that the material which is present in the egg is used, not in its original form but in a modified condition."

The distribution of the nitrogenous compounds in the various stages of development offers the easiest method of attack. In the preceding paper I presented a study of the nitrogen distribution in fresh eggs of the brook trout (*Savelinus fontinalis* L.), and in embryos and young fry at different stages in their development. The present paper is devoted to a somewhat similar study of the eggs and of the newly-hatched larvae of the salamander, *Cryptobranchus Alleghenienis*, Daudin.

The Material.—The eggs of *Cryptobranchus* have proved to be a excellent material for chemical studies. In most amphibian eggs the tough

jell which surrounds the egg makes the preparation of the first sample very difficult, which fact, combined with the usual small size of amphibian eggs, probably accounts for our lack of knowledge of the chemistry of these eggs. Until very recently only a few eggs of *Cryptobranchus* had ever been found but I was fortunate enough to be assisted in my search for the eggs by Dr. Bertram G. Smith, the only person who has ever found them in abundance, and I wish to take this opportunity of thanking him for his many courtesies.

The eggs were secured in the northwestern part of Pennsylvania in the early part of September. Each female deposits about 450 eggs in a hole underneath a rock. The eggs average about 6.2 mm. in diameter are bright yellow in color, a rather deep yellow at the lower pole grading to a pale yellow at the upper pole, and are inclosed in a gelatinous outer envelope about 18 mm. in diameter. These envelopes are joined in a long chain by a thin connecting cord so that the eggs as found resemble a long string of beads. By cutting the envelope with scissors the eggs are easily removed. The vitelline membrane surrounding the yolk mass is very delicate, so that the eggs after removal from the outer envelope must be handled in water with a large mouth pipette to avoid breaking them. For this reason I was unable to secure data as to the weight of the egg before drying. After drying the eggs average about 0.058 g.

The eggs which were secured in advanced stage of development were allowed to remain in shallow dishes just covered with filtered lake water until a part had hatched. Smith (1912) gives the time necessary for development to hatching as about six weeks. Those which had not hatched (but which would have hatched within 48 hours) were released from the envelope by cutting with scissors. The newly-hatched larvae are about 23–25 mm. in length and have a dry weight of approximately 0.057 gram.

Experimental.

The Method.—Sample 1 consisted of 150 eggs in early cleavage stages; sample 2 of 100 larvae at hatching. The eggs and larvae were placed in weighing bottles and dried in a water oven at the temperature of boiling water. Undoubtedly there are objections to this method of drying, but I believe that this is preferable to drying *in vacuo* at a low temperature, since, in the latter, a certain amount of autolysis is almost sure to take place. Autolysis is prevented by both the high temperature and by the rapid drying of a water oven and I do not believe that the nitrogen ratios are altered by such treatment.

After ascertaining the dry weight, the eggs and larvae were removed as completely as possible[1] to an extraction thimble and extracted with anhydrous ether in a Soxhlet apparatus for 48 hours. The ether from the

[1] A very small amount, 0.33% of the total in the case of the eggs and 0.15% in the case of the larvae, persisted in sticking to the walls and sides of the weighing bottles.

eggs became deep golden yellow in color while that from the larvae was colored a yellowish red. Six hours after the extraction began the liquid syphoning was colorless. The ether was evaporated on a water bath and the residue dried to constant weight at 90°. The residue from the eggs was more fluid than that from the larvae and the color differences were the same as those of the ethereal solutions.

The residues remaining in the extraction thimbles were next extracted with absolute alcohol in a Soxhlet apparatus for 48 hrs. After the ether extraction had been completed the residue in the extraction thimble was still bright yellow and this *second yellow pigment* proved readily soluble in alcohol, being all removed at the end of 6 hrs.' extraction, leaving the residue a dull gray. The alcohol was evaporated on a water bath and the residue dried at 90°. This fraction is designated as "ether insoluble but alcohol soluble."

The residue remaining from the alcohol extraction was dried at 90° and weighed, this being the "protein portion" of the eggs and larvae.

The nitrogen partition was determined on the ether soluble and on the ether-insoluble-but-alcohol-soluble portions as follows:

To the residues in the flasks I added 50 cc. HCl of 1.115 sp. g. and boiled the mixture on an electric hot plate for 18 hrs.[1] The HCl was removed, as completely as possible, by distillation on a water bath under a pressure of 20 mm. and 150 cc. of water and 100 cc. of 95% alcohol added to the residue in the flask. An excess of a 10% suspension of $Ca(OH)_2$ was then added and the ammonia nitrogen distilled off into standard acid under a vacuum of 20 mm. at 40–45°.

The mixture remaining in the flask, after the removal of the ammonia nitrogen, was filtered and the residue washed with hot water until chlorides were removed. The nitrogen in the residue on the filter was determined by the Kjeldahl method and recorded as humin nitrogen. In this fraction is included not only the true "humin" nitrogen, but also the nitrogen of any nitrogenous lipin which has not been rendered water soluble by the acid hydrolysis, as well as any basic nitrogenous compound which forms an insoluble calcium salt.

The filtrate from the humin nitrogen was evaporated to a small volume

[1] Of course there are objections to this hydrolysis with acids, but, since the nitrogenous compounds are of the nature of lipins, if they were not hydrolyzed with acid (or alkali) the greater part, if not all, of the nitrogen would be found water insoluble and therefore appearing in the humin nitrogen fraction as a result of adsorption and precipitation with the $Ca(OH)_2$. By acid hydrolysis under constant conditions one should obtain *comparative* results, and after a study of the behavior of the known nitrogenous lipins and other nitrogenous compounds which would be dissolved by either the ether or the alcohol extraction to acid hydrolysis (which study I hope to make soon) it may be possible to determine, to some extent, the nature of the compounds with which we are dealing.

under diminished pressure and then washed into a 200 cc. Erlenmeyer flask. Nine cc. of conc. HCl and sufficient water were added to make the volume 100 cc. and, after heating, 7.5 g. of phosphotungstic acid were added and the mixture set aside in a cool place for 48 hours to permit the basic phosphotungstates to separate. The precipitate was washed as directed by Van Slyke (1911) and the nitrogen content of the bases determined by Kjeldahl.

The entire filtrate from the bases was concentrated in a Kjeldahl flask and the non-basic nitrogen determined by Kjeldahl's method after the addition of 35 cc. conc. H_2SO_4, 15 g. K_2SO_4, and 0.25 g. $CuSO_4$.

The "protein fraction" of the eggs and larvae was analyzed in exact conformance with my modification (as regards dilutions, etc.) of Van Slykes' method (1911, 1912) as already described in THIS JOURNAL (Gortner, 1913), so that a second detailed description is unnecessary.

The analytical data follow:

Sample 1.—150 eggs removed from the uterus and fertilized artificially at 4:30 p.m. Sept. 6th. These eggs were allowed to develop until 3 p.m. Sept. 9th, when they were carefully removed from their envelopes and the drying was begun.[1]

Weight of 150 eggs dry at 100° = 8.7381 g.

Ether soluble portion = 1.6772 g.

Nitrogen in ether-soluble portion = 0.00378 g. divided as follows:

Ammonia N = 0.30 cc. 0.1 *N* acid indicating 0.00028 g. N in 100 eggs.

Humin N = 1.20 cc. 0.1 *N* acid indicating 0.00112 g. N in 100 eggs.

Basic N = 0.30 cc. 0.1 *N* acid indicating 0.00028 g. N in 100 eggs.

Non-basic N = 0.90 cc. 0.1 *N* acid indicating 0.00084 g. N in 100 eggs

Alcohol-soluble-but-ether-insoluble-portion = 0.9945 *g.*

Nitrogen in alcohol fraction = 0.0105 g., divided as follows:

Ammonia N = 0.15 cc. 0.1 *N* acid indicating 0.00014 g. N in 100 eggs.

Humin N = 1.80 cc. 0.1 *N* acid indicating 0.00168 g. N in 100 eggs.

Basic N = 4.10 cc. 0.1 *N* acid indicating 0.00382 g. N in 100 eggs.

Non-basic N = 1.45 cc. 0.1 *N* acid indicating 0.00136 g. N in 100 eggs.

Protein fraction (insoluble in ether or alcohol) = 6.0385 *g.*[2]

Nitrogen in the protein fraction = 0.8631 g., distributed as follows:

Ammonia N = 62.4 cc. 0.1 *N* acid indicating 0.0582 g. N in 100 eggs.

Humin N = 14.1 cc. 0.1 *N* acid indicating 0.0132 g. N in 100 eggs.

[1] It was impossible to begin the drying at an earlier stage because of the necessity of transporting the material from western Pennsylvania to the laboratory. There should be some delay before eggs are taken for development studies in order to see whether the eggs are fertile and would develop. The eggs forming my sample were probably in Stage 8 (Smith 1912) and had developed to probably 256 cells, perhaps slightly more. It is highly improbable that this amount of development could have caused any chemical changes large enough to be detected by my methods of analysis.

[2] A slight loss occurred in removing the dried eggs from the weighing bottle to the extraction thimble so that the ether + alcohol + protein fractions only total 99.67% of the dry weight.

The filtrate from the humin was made to 200 cc. and nitrogen in the filtrate from the humin determined on 10 cc. portions, (1) 26.90 and (2) 27.10 cc. 0.1 *N* acid indicating 0.5040 g. N in the filtrate from the humin in 100 eggs.

The bases were precipitated by the addition of 18 cc. conc. HCl and 15 g. of phosphotungstic acid to 160 cc. of the above solution.

Arginine N (on $^1/_2$ of the bases) = 17.0 cc. 0.1 *N* acid indicating 0.0793 g. N in 100 eggs.

Total basic N (on $^1/_2$ bases + arginine) = 74.15 cc. 0.1 *N* acid indicating 0.1730 g. N in 100 eggs.

Amino N in bases (from 5 cc.) = 19.25 cc. N at 23° and 768 mm. (from 7.5 cc.) = 29.2 cc. N at 22° and 768 mm. indicating 0.0916 g. amino N in the bases of 100 eggs.

Cystine N (on 10 cc.) = 0.0050 g. $BaSO_4$ indicating 0.00126 g. N in 100 eggs.

Histidine N (calc.) = 0.0329 g. in 100 eggs.

Lysine N (calc.) = 0.0595 g. in 100 eggs.

Nitrogen in the filtrate from the bases (made to 200 cc.) = (on 25 cc. portions) 35.20 and 35.60 cc. 0.1 *N* acid indicating 0.3304 g. N in 100 eggs.

Amino N in the filtrate from the bases = (on 10 cc.) 33.9 cc. N at 21.5° and 749 mm. and 33.8 cc. N at 21.5° and 750 mm. indicating 0.3142 g. amino N in 100 eggs.

These data calculated to *per cent. of the total nitrogen of the egg* form the fourth column, and the *weight of nitrogen in g.* forms the first column in Table II.

Sample II.—100 larvae at hatching. Some had just hatched while others were released from the membrane.

Weight of 100 larvae dry at 100° = 5.7285 g.

Ether soluble portion = 1.2747 g.

Nitrogen in ether soluble portion = 0.00455 g. divided as follows:

Ammonia N = 0.45 cc. 0.1 *N* acid indicating 0.00063 g. N.

Humin N = 1.60 cc. 0.1 *N* acid indicating 0.00224 g. N.

Basic N = 0.20 cc. 0.1 *N* acid indicating 0.00028 g. N.

Non-basic N = 1.0 cc. 0.1 *N* acid indicating 0.0014 g. N.

Alcohol-soluble-but-ether-insoluble portion = 0.6179 g.

Nitrogen in alcohol soluble portion = 0.0111 g. distributed as follows:

Ammonia N = 0.10 cc. *N* acid indicating 0.00014 g. N.

Humin N = 1.70 cc. 0.1 *N* acid indicating 0.00238 g. N.

Basic N = 3.50 cc. 0.1 *N* acid indicating 0.0049 g. N.

Non-basic N = 2.65 cc. 0.1 *N* acid indicating 0.0037 g. N.

Protein residue (insoluble in alcohol and ether) = 3.8278 g.[1]

Nitrogen in the protein residue = 0.5688 g., distributed as follows:

Ammonia N = 42.60 cc. 0.1 *N* acid indicating 0.0596 g. N.

Humin N = 9.70 cc. 0.1 *N* acid indicating 0.0136 g. N.

The filtrate from the humin was made to 200 cc. and the nitrogen content determined on 10 cc. portions = 17.7 and 17.7 cc. 0.1 *N* acid, indicating 0.4956 g. *N* in the filtrate from the humin.

[1] The ether + alcohol + protein fractions total only 99.85% of the dry weight because of a slight loss which occurred by incomplete removal from the weighing bottle.

The bases were precipitated from 160 cc. of the above solution, and were finally made to 50 cc. volume.

Arginine N (on 1/2 bases) = 11.6 cc. 0.1 *N* acid indicating 0.0812 g. N.

Total N in the bases (on 1/2 bases + arginine N) = 49.05 cc. 0.1 *N* acid indicating 0.1717 g. N.

Amino N in **bases** = (on 5 cc.) 13.10 cc. N at 23° and 768 mm. and (on 8.5 cc.) 21.5 cc. N at 24° and 769 mm. indicating 0.0911 g. N.

Cystine N = 0.0044 g. $BaSO_4$ (on 10 cc.) indicating 0.00165 g. N.

Histidine N (calc.) = 0.0288 g. N.

Lysine N (calc.) = 0.0600 g. N.

The filtrate from the bases was made to 200 cc. and nitrogen found on 25 cc. portions = 22.80 and 22.80 cc. 0.1 *N* acid indicating 0.3192 g. N.

Amino N in the filtrate from the bases = (on 10 cc.) 21.3 cc. N at 21° and 764 mm. and 21.3 cc. N at 20.5° and 763 mm. indicating 0.3035 g. N.

These data calculated to *per cent. of the total nitrogen* in the larvae form the fifth column and the *percentage change from egg to larvae* forms the sixth column in Table II. *The weight of the nitrogen in grams* per 100 larvae forms the second column and the *change of nitrogen* in grams in the development of the egg to the hatching stage forms the third column in Table II.

TABLE I.—COMPARATIVE ANALYSES OF CRYPTOBRANCHUS EGGS AND OF THE LARVAE AT HATCHING.

	Weight in grams.			Percentages.		
	100 eggs.	100 larvae.	Change.	Eggs.	Larvae.	Change.
Dry weight	5.8254	5.7285	—0.0969	...	...	—1.66%
Ether sol	1.1182	1.2747	+0.1565	19.19	22.25	+3.06
Ether insol. but alcohol sol	0.6630	0.6179	—0.0451	11.38	10.78	—0.60
Protein	4.0256	3.8278	—0.1978	69.10	66.82	—2.28
N content	0.5849	0.5845	—0.0004	...	...	.
N in ether	0.00252	0.00455	+0.00203	0.44	0.78	+0.34
N in alcohol	0.0070	0.0111	+0.0041	1.20	1.90	+0.70
N in protein	0.5754	0.5688	—0.0066	98.37	97.30	—1.04
% N in protein fraction	...	...	...	14.30	14.86	+0.56

Discussion.

Although the analyses recorded in the preceding tables agree in most entries so well that we could conclude that the samples analyzed were duplicates, there are a few entries which show changes which are certainly significant. A careful study of these data has convinced me that all, or nearly all, of the nitrogen ratios are altered to some extent as embryonic growth progresses, but that the monoamino acids are the more generally utilized. Such a conclusion is in excellent agreement with my earlier findings (Gortner, 1913).

During the development there was a loss of 1.66% of the dry weight (0.0969 g. per 100 larvae) which must be attributed to carbon dioxide and water, inasmuch as no nitrogen was lost during this period of growth.

TABLE II.—DISTRIBUTION OF NITROGEN IN 100 CRYPTOBRANCHUS EGGS AND LARVAE.

		In grams.			In percentage.		
		Eggs.	Larvae.	Change.	Eggs.	Larvae.	Change.
Ether soluble	Ammonia N	0.00028	0.00063	+0.00035	0.048	0.108	+0.06
	Humin N	0.00112	0.00224	+0.00112	0.191	0.383	+0.192
	Basic N	0.00028	0.00028	...	0.048	0.048	...
	Non-basic N	0.00084	0.00140	+0.00056	0.114	0.239	+0.125
Ether insoluble, alcohol soluble	Ammonia N	0.00014	0.00014	...	0.024	0.024	...
	Humin N	0.00168	0.00238	+0.00070	0.287	0.407	+0.120
	Basic N	0.00382	0.00490	+0.00108	0.654	0.838	+0.184
	Non-basic N	0.00136	0.00371	+0.00235	0.231	0.635	+0.404
Protein fraction	Ammonia N	0.05824	0.05964	+0.0014	9.956	10.21	+0.254
	Humin N	0.01316	0.01358	+0.00042	2.25	2.324	+0.074
	Basic N	0.1730	0.1717	—0.0013	..	..	...
	Non-basic N	0.3304	0.3192	—0.0112	..	..	...
	Arginine N	0.07932	0.08121	+0.0019	13.56	13.90	+0.34
	Cystine N	0.00126	0.00165	+0.0004	0.213	0.282	+0.07
	Lysine N	0.05946	0.0600	+0.00054	10.16	10.27	+0.11
	Histidine N	0.03292	0.0288	—0.0041	5.63	4.93	—0.70
	Amino N filt. from bases	0.3142	0.3035	—0.0107	53.73	51.92	—1.81
	Non-NH_2 N in filt	0.0162	0.0157	—0.0005	2.76	2.686	—0.074
	Total N in samples	0.58492	0.58450	—0.00042	..	..	...
	N recovered in analyses	0.5843	0.57976	...	99.856	99.204	...
	Loss of N in analyses	0.00062	0.00474	...	..	..	...

Accompanying this loss in weight there is a very marked *gain of fats* equal to 3.06% of the egg weight and to an increase of 14% of the fat already present in the egg.

Tangl and Farkas (1904) have observed a similar synthesis of fat in developing trout embryos and explain this finding by the assumption that there are present, in the egg, glycoproteins which are broken down to carbon dioxide, water, glycogen, fat, and "all of the nitrogen of the protein is retained in the organism in the form of urea (or uric acid)," the difference in energy content being the "Entwicklungsarbeit." I believe that their hypothesis is in part correct, *i. e.*, that the energy of development comes from a carbohydrate nucleus, but other parts of their suggestion I find to be erroneous.

Tangl and Farkas submit no proof for their contention that the nitrogen of the protein is converted into urea (or uric acid). In discussing this phase of their work in my earlier paper, I showed that, in all probability, no considerable quantity of either urea or uric acid was present in the young trout at hatching and that, therefore, their hypothesis did not hold in its entirety. In this series of analyses of *Cryptobranchus* eggs and larvae there can be no doubt that no urea is formed and in all probability none, or at most only a trace, of the nitrogen is converted into uric acid.

The behavior of urea and of uric acid to extraction with ether and alcohol was first investigated. The statement that urea is insoluble in ether and that uric acid is insoluble in both ether and alcohol seems to find no exception in the solubility literature. As I have shown elsewhere, (Gortner 1914), *this statement is not true*, for although urea is practically insoluble (approx. 0.0004 g. in 100 cc.) in anhydrous ether nevertheless *as much as 0.0720 g. is dissolved in the course of a 48-hour extraction*, and while uric acid is still more insoluble in absolute alcohol (approx. 0.00008 g. in 100 cc.), *as much as 0.0260 g. is extracted in 48 hours* in a Soxhlet apparatus.

We should look, therefore, for an increase in *Ammonia N* in the ether soluble fraction if urea has been formed within the organism, and the observed increase, while possibly significant, is only 0.3% of the required amount.[1] The entire gain of ammonia N in all three fractions analyzed would be only 1.6% of the expected urea N.

Uric acid would be extracted, at least in part, by the absolute alcohol and an increase in ammonia N should follow a synthesis of uric acid, since I have shown that 15% of the uric acid nitrogen is split off as ammonia

[1] Tangl and Farkas found a gain of 0.38 g. of fat and an energy loss of 3.46 kg. Cal. in the course of the development of 518 trout eggs and theorize that 1.67 g. of glycoprotein (9.7 Cal.) must be broken down to 0.38 g. fat (3.5 Cal.), 0.30 g. glycogen (1.3 Cal.) and 0.57 g. urea (1.40 Cal.) the difference in calculated heat values being 3.5 Cal. Using their figures I should find 0.234 g. of urea containing 0.109 g. N due to a synthesis of 0.1565 g. fat.

by acid hydrolysis (Gortner 1913, p. 642). There is no gain in ammonia N in the alcohol extract and the total gain of N in this fraction is only an insignificant part of the theoretical uric acid nitrogen. *We must therefore conclude that no appreciable amount of urea or of uric acid is formed in Cryptobranchus during embryonic growth.*

The analyses show, further, that the origin of the fats synthesized is, in a large measure at least, to be found in the protein fraction but that *it is not a simple protein which has been altered*, for, although there is a loss of 0.1978 g. from the protein fraction, the nitrogen content of the lost portion is only 3.33%, leaving a remaining protein residue with a relatively increased nitrogen content.

The above facts would seem to support only one argument, *i. e.*, that there is present in the eggs of *Cryptobranchus* a carbohydrate nucleus either free (glycogen) or more probably in the form of a glycoprotein (ovomucoid?). During the process of development this carbohydrate is broken down to carbon dioxide and water with the consequent releasing of energy for the "Entwicklungsarbeit," but the breaking down of the carbohydrate proceeds more rapidly than is necessary to provide the energy of development and, as a result, the surplus energy is stored in the form of fat. If the carbohydrate is a part of a protein molecule, the amino acids are not "burned" but are utilized in part in their original form and in part to furnish the nitrogen for lecithin and other nitrogenous compounds necessary for the development of the growing embryo.

Several additional points demand brief comments. There is a gain of nitrogen in both the ether soluble and the ether-insoluble-but-alcohol-soluble fractions. It seems possible that the gain in the ether soluble fraction is due to a synthesis of lecithins such as Tichmiroff (1885) observed in developing eggs of the silk worm (*Bombyx mori*). It is very evident from the nitrogen partition that the nitrogen in the alcohol extract is all, or nearly all, from basic nitrogenous compounds, probably pyrimidine or purine bases. Kossel (1886) and Mendel and Leavenworth (1908) have shown that purine bases are synthesized as embryonic growth progresses in hen and duck eggs. Unfortunately we know too little of the solubilities of the purine and pyrimidine bases *under conditions of continuous extraction* to decide which compounds would be found in the ether extract, the alcohol extract, or which should be insoluble in both alcohol and ether.

One surprising result (to me) of these analyses was the large percentage of "ether insoluble but alcohol soluble" compounds in the egg. I know of no data dealing with this fraction (as regards non-nitrogenous compounds) and should consider it a great favor if any reader could call to my attention such data as may exist or suggest the possible nature of these substances.

Summary.

1. The eggs of *Cryptobranchus* contain two yellow pigments, one being soluble in ether and the other insoluble in ether but soluble in absolute alcohol.

2. There is a total loss of dry weight (CO_2 and H_2O) during the development of 100 eggs to the hatching stage of 0.0969 g. or 1.66% of the dry weight.

3. There is a *gain* of fats during development of 0.1565 g. per 100 eggs this being equal to an increase of 14% of the fat already present in the egg.

4. There is a loss of 0.0451 g. from the fraction insoluble in ether but soluble in absolute alcohol. The significance of this loss is unknown.

5. There is a loss of 0.1978 g. from the protein fraction, but in all probability this is not a loss of a simple protein but of a carbohydrate radical which has been broken down to form fat, etc.

6. There is neither loss or gain of total nitrogen during development.

7. There is a gain of nitrogen in the ether soluble portion of 0.00203 g. This would indicate a synthesis of 0.1174 g. of di-steryl-lecithin (1.73% N), providing all of the nitrogen in the ether soluble portion is counted as lecithin.

8. There is a gain of 0.0041 g. of nitrogen in the ether-insoluble-but-alcohol-soluble fraction. The nitrogen in this fraction is largely basic nitrogen, probably purine or pyrimidine bases.

9. There is a loss of 0.0066 g. nitrogen from the protein fraction. Nearly all of this loss comes from the mon-amino acids.

10. There is considerable evidence that the nitrogen ratios in the protein fraction are not fixed quantities but that some amino acids are more necessary for the development of the embryo than are others and as a result there is a continuous breaking down and recombining of the resulting radicals into new compounds. For example, the gain observed for Arginine N, although small, is probably significant.

11. No appreciable amount of either urea or of uric acid is formed during embryonic development of *Cryptobranchus*.

12. It seems probable that there is, in the eggs of *Cryptobranchus*, a carbohydrate nucleus either free (glycogen) or combined in the form of a glycoprotein and that during the process of embryonic growth this carbohydrate is broken down to carbon dioxide and water with a consequent liberation of energy for the "Entwicklungsarbeit," but the breaking down of the carbohydrate proceeds more rapidly than the needs of growth demand, with the result that the surplus energy is stored as fat.

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COLD SPRING HARBOR,
LONG ISLAND, N. Y.

THE INVERSION OF SUCROSE BY INVERTASE. VIII. AN IMPROVED METHOD FOR PREPARING STRONG INVERTASE SOLUTIONS FROM TOP OR BOTTOM YEAST.

BY C. S. HUDSON.[1]

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The Clerget method for estimating sucrose depends upon the measurement of the change in optical rotation which accompanies the hydrolysis of the sugar. Hydrochloric acid is generally used as the hydrolyst although it is known to hydrolyze inulin, raffinose, and some other carbohydrates under the mildest conditions that will accomplish the inversion of sucrose.

In 1881, it was proposed by Kjeldahl[2] to use the enzyme invertase in place of hydrochloric acid in the Clerget method because invertase was regarded as a more nearly specific hydrolyst for sucrose. Although it is now understood that invertase hydrolyzes raffinose, gentianose, and stachyose as well, it seems that these sugars are derivatives of sucrose and that invertase may still be strictly regarded as a specific hydrolyst of sucrose and certain of its immediate derivatives. Kjeldahl used, in one procedure, an aqueous extract of yeast to hydrolyze sucrose, and, in an alternative one, a portion of yeast added to the sucrose solution which was kept at 52° with a little thymol added to prevent alcoholic fermentation. As invertase is rapidly, though incompletely, extracted from yeast by water, its action can be obtained by either of these procedures. Kjeldahl's untimely death prevented the further development of this excellent analytical

[1] Contribution from the Division of Carbohydrate Investigations, Bureau of Chemistry.

[2] Compt. rend. Carlsberg Laboratoire, *Copenhagen*, 1, 189–95 (1881).