$\frac{1}{2}$-inch stage. It is thus much later in developing than in *Salmo*;* and the fold forming its front wall never extends backwards to the same degree as in that form and in *Anarrhicas*. This fold, in the post-larval *Zoarces*, is thickened in its apex, and lodges a fine commissure. As pointed out by Balfour in Elasmobranchs the fold is due to the upward rotation of the cerebrum.

The fibrous tract over the 3rd ventricle in the herring is well marked in the $\frac{3}{4}$-inch stage. It is seen to consist of fibres passing upwards and inwards from the optic thalami to the middle line above the 3rd ventricle, and then running forward to the stalk of the pineal body. The tract has a double nature, as is readily seen in vertical longitudinal sections of a herring $1\frac{1}{2}$ inch long. It is seen here to be a backwardly directed fold of the brain roof, continuous ventrally with the back wall of the pineal stalk, and dorsally with the roof of the optic ventricle, the apex of the fold being the posterior commissure. Its length in this form is due to the flattening of the brain, the tract being very short in *Zoarces*, where the brain is not flattened. In *Zoarces*, also, from the same cause, the limbs of the fold are less closely applied to each other and much thicker.

The pineal body is roundish and solid in the early larval stage in the herring. It is vertically flattened in the early post-larval stage. In the $\frac{1}{2}$-inch stage it is much larger and contains a lumen; it shows signs of constriction into proximal and distal elements, and the lumen contains a coagulable albuminous fluid, as in *Petromyzon*.† In the $1\frac{1}{2}$-inch stage the constriction is still visible, and the walls are generally crenated. The tissues of the pineal wall are now divided into three layers, and are of varying thickness. The cartilage of the tegumen cranii overlies the body at this stage. The constriction of the body appears to be an exaggeration of the crenation of the pineal wall met with in *Salmo*; it has not, probably, the morphological value of the constriction of the body in *Petromyzon*.

IV. "A Cyanogen Reaction of Proteids." By J. Gnezda, M.D. Communicated by Professor E. A. Schäfer, F.R.S. (From the Physiological Laboratory, University College, London.) Received December 19, 1889.

When dry urea is heated to its melting point it gives off ammonia, and a substance called biuret ($C_2N_3H_5O_2$) remains behind. Biuret is decomposed by heat into ammonia and cyanuric acid ($C_3N_3H_3O_3$).

* Hoffmann, "Zur Ontogenie der Knochenfische," 'Arch. Mikrosk. Anat.,' vol. 23, 1884.

† Beard, "Parietal Eye in Cyclostomatous Fishes," 'Quart. Journ. Micros. Sci.,' 1889.

G. Wiedemann* discovered that on adding an alkaline solution of copper sulphate to cyanuric acid, a violet solution was produced. The same investigation showed that biuret gave a rose-red solution on treatment with copper sulphate and sodium hydrate.

One of the ordinary tests for a proteid, which is sometimes called Piotrowski's reaction, is the violet solution produced by adding copper sulphate and caustic potash or soda. Albumoses and peptones differ from other proteids in giving with these reagents a rose-red instead of a violet solution; the colour so produced is the same as that given by biuret, hence this reaction is generally spoken of as the *biuret reaction*. It is stated that after heating any proteid with caustic soda, and then adding copper sulphate, the rose-red coloration of the biuret reaction appears; and this is undoubtedly the case, for the action of the hot concentrated alkali is to form not only alkali-albumin, but also some substances of the albumose class. The whole value of the test in distinguishing between native albumins on the one hand, and peptones and albumoses on the other, depends on its being performed in the cold.

Brücke† especially has emphasised the difference between the violet coloration given by ordinary proteids and the rose-red (so-called biuret) reaction of the peptones. He however considers that the radicle in the complex proteid molecule which gives rise to the reaction is probably the same in both cases, but that it is some other change in the molecule that causes the difference of tint.

Salkowski‡ has recently investigated the colour reactions of the proteids, and shown that Millon's reaction and the Adamkiewicz reaction are produced by the presence in the proteid molecule of certain aromatic radicles. He does not, however, appear to have investigated the biuret reaction; and the object of my own work has been to discover, if possible, upon what groups of atoms in the proteid molecule this reaction depends. Whether the violet colour of ordinary proteids is due to cyanuric acid, and the rose-red colour of peptones to biuret, I am, however, unable to say. The main result of my experiments has been that these reactions depend on the presence in a proteid of cyanogen, or of some cyanogen-containing radicle. I have also, by means of somewhat modifying the method usually adopted for performing the test, discovered that it may be used for distinguishing classes of proteids from one another more accurately than has hitherto been possible.

The chief modification I have introduced into the test has been the addition of ammonia, either instead of or in addition to the potash or

* 'Poggendorff's Annalen,' vol. 74, 1849, p. 67.

† *Sitzungsberichte* of the Vienna Academy, 1883, reprinted in 'Monatshefte f. Chemie,' vol. 4.

‡ 'Zeitsch. f. Physiol. Chem.,' vol. 12.

soda usually employed. In some cases I have added each reagent separately, first copper sulphate, then ammonia, and then potash or soda; but, as a rule, I have employed a reagent made by dissolving a copper salt in ammonia; the dark blue solution that results is an ammoniacal solution of cupric hydroxide. After adding this to a solution of proteid and observing the result, potash or soda can be added subsequently. The use of the ammoniacal solution of cupric hydroxide as a reagent for detecting proteids was first suggested to me by the fact that on adding some of it to urine from a case of cystitis I obtained a reddish-violet colour. In the light of subsequent experiments, it is probable that this urine contained a peptone or peptone-like substance derived from the decomposition of pus corpuscles.

The reactions I obtained with various proteids may be thus tabulated:—

Proteids.	Addition of ammoniacal solution of cupric hydroxide produced:—	Subsequent addition of potassium or sodium hydroxide produced:—
Egg albumin.	Pale blue solution.	Violet solution.
Serum albumin.	Pale blue solution.	Violet solution.
Grübler's peptone.	Violet solution.	Rose-red solution.
Witte's peptone.	Violet solution.	Rose-red solution.
Pure peptone (prepared from Witte's peptone).	Violet solution.	Rose-red solution.
Albumoses (prepared from Witte's peptone).	Violet solution.	Rose-red solution.

Native albumins differ from the products of proteolysis (albumoses and peptones) by giving no change of colour with an ammoniacal solution of cupric hydroxide; when potash or soda is subsequently added, the result is, as usual, a violet solution. The albumoses and peptones, on the other hand, give a violet solution with the ammoniacal cupric hydroxide; this is turned red on the subsequent addition of potash or soda.

Copper sulphate and ammonia added separately give the same results. When a drop of copper sulphate solution is added to a proteid solution, the result is a precipitate of an albuminate of copper;* on adding ammonia, this dissolves up; if the solution is blue, changing to violet when potash is added, albumoses and peptones are absent; but if the solution is violet, changing to red when potash is added, albumoses or peptones, or both, are present.

* This preliminary precipitation does not, however, occur with deutero-albumose nor with pure peptone.

This method of performing the test has a great advantage over the way in which it is usually done, as it is much easier to distinguish between the blue of the dissolved cupric hydroxide and the violet due to peptone when ammonia is added than it is between the violet solution given by albumin and the rose-red given by peptones when potash is added, especially if the solutions be dilute. The test with ammonia has also this advantage, that peptones and albumoses can be detected with certainty even if other proteids are present at the same time.

I next proceeded to make experiments with nickel sulphate dissolved in ammonia; the solution so formed is a purplish one, and the results obtained may be tabulated as before:—

Proteid.	Addition of nickel oxide in ammonia produced:—	Subsequent addition of potassium or sodium hydroxide produced:—
Egg albumin.	Faint bluish solution.	Yellow solution.
Serum albumin.	Faint bluish solution.	Yellow solution (with flocculent precipitate).
Witte's peptone.	Yellow solution (with flocculent precipitate).	Orange solution (with flocculent precipitate).
Grübler's peptone.	Ditto.	Ditto.
Pure peptone.	Ditto.	Ditto.
Albumoses.	Ditto.	Ditto.

This test may thus be used for distinguishing between albumins and the products of proteolytic digestion; the former giving a yellow solution only after the addition of potash or soda, the latter giving a yellow colour with nickel oxide and ammonia alone, which is however deepened to a dull orange by the addition of potash or soda.

I next proceeded to apply these tests to other classes of proteids, albuminates, globulins, fibrin, coagulated proteid, and mucin; and the results are stated in the following table:—

Proteid.	Addition of cupric hydroxide in ammonia produced :—	Potash or soda then added produced :—	Nickel oxide in ammonia produced :—	Potash or soda then added produced :—
*Albuminates.**				
Acid albumin.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.
Alkali albumin.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.
Casein.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.
Globulins.†				
Serum globulin.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.
Vitellin.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.
Myosin.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.
Fibrin.	Blue.	Violet.	Pale blue.	Yellow.
Coagulated proteid.	Violet.	Rose-red.	Yellow.	Orange.
Mucin.	Blue solution.	Violet solution.	Pale blue solution.	Yellow solution.

* The acid albumin and alkali albumin were prepared from egg albumin by adding to it a few drops of very dilute acid and alkali respectively, and warming to 40° C. for 10—15 minutes.

† Dissolved in 1 per cent. ammonium sulphate solution or a dilute magnesium sulphate solution.

Coagulated proteid behaves like the peptones; this is easily explicable, as Neumeister* has shown that the action of hot water on proteids forms from them small quantities of albumoses due to hydration. The other proteids in the above list behave like the albumins, and thus differ from the albumoses and peptones.

I now pass from the proteids to certain derivatives of proteids, these experiments being designed to elucidate the question as to what radicle it is on the proteid molecule to which these reactions are due.

Uric Acid—Uric acid was dissolved in soda and boiled; then cooled, and a drop of copper sulphate added; this did not colour the fluid at all; on adding more, a violet solution was obtained.†

Uric acid dissolved in soda, but not heated, gave with cupric hydroxide in ammonia a pink colour.

A little uric acid was evaporated to dryness on a porcelain dish with nitric acid; on adding cupric hydroxide in ammonia to this a violet colour is obtained; this is not simply the murexide test produced by the ammonia, as nickel oxide dissolved in ammonia gives a deep yellow colour. Thus uric acid gives the reactions very much as proteids do.

Xanthine, *hypoxanthine*, and *sarcosine* give the same reactions.

Biuret and *cyanuric acid* are the substances in which the colour tests with cupric hydroxide in alkaline solutions were first observed; the following are the particulars of the experiments I have performed with these two substances:—

Addition of—	To aqueous solution of biuret.	To aqueous solution of cyanuric acid.
(1.) Cupric sulphate.	No effect.	No effect.
(2.) Cupric sulphate and potash.	Rose-red solution.	Violet solution.
(3.) Cupric sulphate and ammonia.	Blue solution.	Blue solution.
(4.) Cupric sulphate and ammonia, followed by potash.	Rose-red solution.	Violet solution.
(5.) Nickel sulphate dissolved in ammonia.	Blue solution.	Blue solution.
(6.) Nickel sulphate in ammonia, followed by potash.	Orange solution (with flocculent precipitate).	Yellow solution (with flocculent precipitate)

* 'Zeitsch. f. Biol.,' vol. 24, p. 272.

† Winogradoff ('Virchow's Archiv,' vol. 27, p. 565) and Worm-Müller ('Pflüger's Archiv,' vol. 27, p. 31) mention something similar, but not in connexion with the present subject.

Biuret thus behaves in very much, but not exactly, the same way as peptones; while cyanuric acid gives the same colour reactions as albumin.

Hydrocyanic Acid.—The same series of reactions was tried with this substance, and the result was that the colours obtained were precisely the same as those obtained with peptones and albumoses. The details are as follows:—

Addition of	Produced
Cupric sulphate	No effect.
Cupric sulphate and potash	Rose-red solution.
Cupric sulphate and ammonia, or ammoniacal solution of cupric hydroxide	Violet solution.
Subsequent addition of potash or soda.....	Rose-red solution.
Nickel sulphate dissolved in ammonia.....	Yellow solution.
Subsequent addition of potash or soda.....	Orange solution.

The colours produced are in some cases evanescent, and, if any free acid is left not neutralised by the ammonia or potash added, the liquid remains colourless.

Glycocine, *Leucine*, *Tyrosine*.—These substances gave negative results, the liquid remaining blue or bluish-green throughout.

Ethyl aldehyde, *propyl aldehyde*, *valeraldehyde*, *isobutyl aldehyde*, and *benzyl aldehyde* similarly gave entirely negative results.

General Conclusions.

The addition of cupric sulphate and potash to albumin or globulin produces a violet solution. The addition of the same reagents to peptones or albumoses causes a rose-red solution. If ammonia be added as well the results are as follows:—Cupric sulphate and ammonia added to albumin causes a blue solution, turned violet on the addition of potash; cupric sulphate and ammonia added to peptone or albumose gives a violet solution, turned red on the addition of potash.

By this reaction, and by a somewhat similar reaction in which nickel sulphate is used, peptones and albumoses may be easily distinguished from, and detected in the presence of, albumins and globulins.

Not only proteids, but other organic substances ultimately obtainable from proteids, give very similar reactions.

The reaction is not given by the amido-acids, glycocine, and leucine; nor by derivatives, like tyrosine and benzyl aldehyde, that contain an aromatic nucleus, nor by the aldehydes of various alcohols. But in the substances that do give the test, the nitrogen is either partly or wholly combined in the form of cyanogen: these substances are biuret, cyanuric acid, uric acid, xanthine, hypoxanthine, sarco-

Reagent added.	Colour of resulting solutions with				
	Albumins and globulins.	Peptones and albumoses.	Biuret.	Cyanuric acid.	Hydrocyanic acid.
Copper sulphate + ammonia	Blue.	Violet.	Blue.	Blue.	Violet.
Copper sulphate + potash	Violet.	Rose-red.	Rose-red.	Violet.	Rose-red.
Copper sulphate + ammonia + potash.....	Violet.	Rose-red.	Rose-red.	Violet.	Rose-red.
Cupric hydroxide dissolved in ammonia.....	Blue.	Violet.	Blue.	Blue.	Violet.
Cupric hydroxide dissolved in ammonia + potash	Violet.	Rose-red.	Rose-red.	Violet.	Rose-red.
Nickel sulphate dissolved in ammonia	Blue.	Yellow.	Blue.	Blue.	Yellow.
Nickel sulphate dissolved in ammonia + potash	Yellow.	Orange.	Orange.	Yellow.	Orange.

sine, and hydrocyanic acid. It thus appears probable that the colour reaction of the proteids that occurs on addition of a cupric salt and an alkali is due to the existence in the proteid also of cyanogen.

Just as some proteids give a rose-red colour, and others a violet, so, in the list of substances just enumerated, some give a rose-red, and some a violet. Biuret was the substance in which a rose-red colour was first noted; hence the term biuret reaction, as applied to peptones. Cyanuric acid was the substance in which a violet colour was first noted. Probably in both cases the reaction is due to a cyanogen radicle; but the cause of the difference in colour is unknown. In the same way, our ignorance of the constitution of the proteid molecule stands in the way of our discovering the difference between peptones that give a rose-red colour and albumins that give a violet colour.

The term biuret reaction is to some extent a misnomer, as applied to the peptones and albumoses; the test with the modification I have introduced behaves a little differently in the two cases. The substance that peptone most nearly resembles in its colour reactions is hydrocyanic acid, as is shown in the table (p. 209), in which a contrast is drawn between the chief substances which I have examined.

Using the word cyanogen in the widest possible sense, the conclusion I should draw from such a series of experiments is that the colour reaction with a cupric salt and an alkali is a cyanogen reaction. Among the simpler organic bodies examined, we have certain cyanogen compounds, like cyanuric acid, that give a violet colour; and certain proteids (the albumins and globulins) give the same colour. There are certain other substances, like biuret, which give a red colour without any intermediate violet stage; there are others, like hydrocyanic acid, which give a violet colour with ammonia, which is turned red by potash or soda; and to this last group the peptones also belong. Just as there is a different combination of the cyanogen in cyanuric acid from that in hydrocyanic acid, so there is probably the same difference between the combination of the cyanogen in albumin and peptone respectively; and this difference is, as a rule, brought about by a digestive ferment.

Presents, February 20, 1890.

Transactions.

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