uppermost portion of the left motor area. The outer surface of the skull was intact. The opening of the lacerated dura was enlarged, the coagulated blood removed and the cortex exposed. The latter's surface was found intact; no laceration could be seen.

Result.—The patient gradually improved and finally recovered. The mental hebetude completely disappeared. During the first two days the knee-jerk remained slightly exaggerated, but on the fourth day became normal. On the sixth day, the paradoxical reflex became fainter and on the twelfth day after the operation it could no more be elicited. It was interesting to observe its gradual diminution and disappearance and it was also interesting to notice its conspicuous presence on the morning before the operation and its gradual obliteration after the operation. The patient was subsequently seen by me several times during a period of two months and at no time could I detect the presence of an abnormal knee-jerk or of the paradoxical reflex.

This case, I believe, is the most striking illustration of my contention concerning the practical value of the reflex. Added to the first two cases published in 1906 and 1907,³ it furnishes decisive anatomic proofs for the *a priori* deductions made from clinical observations. The reflex has its diagnostic value and deserves a place in nosology of the nervous system.

In conclusion, I may mention that a systematic investigation was made by me in 800 individuals free from diseases of the nervous system, also in 250 cases of functional nervous diseases and at no time was extension of the toes elicited by the method employed by me for the detection of the paradoxical reflex.

1430 Pine Street.

THE AUTOLYSIS OF THE CRYSTALLINE LENS

A PRELIMINARY REPORT

C. A. CLAPP, M.D.
BALTIMORE

In the study of traumatic cataract and the needling operation, I was struck with the very scant description of the manner in which the lens becomes opaque and the causes for its opacity and final absorption.

Parsons¹ states that after exposure of the lens fibers to the action of the aqueous they swell up, become opaque, protrude through the capsular wound, and finally break up in the usual manner, absorption being largely due to the leukocytes, which become swollen and filled with granules.

Schlösser² (1887) in an article on traumatic cataract, and Schirmer³ (1889) have studied the microscopic changes very thoroughly, but without throwing much light on the cause of these changes.

Fuchs⁴ states that the fibers swell up and become opaque through absorption of water; some are broken off and drop into the anterior chamber and are absorbed. But he also says that concussion without rupture of the capsule may cause the development of complete opacity of the lens. He, however, fails to state just why this occurs.

De Schweinitz⁵ says that the opacity and liquefaction are explained by the action of the sodium chlorid of the aqueous on the globulin of the lens substance, as globulin is normally soluble in weak sodium chlorid solution.

This theory was, I think, first suggested by Marcus Gunn in 1899 in the *Ophthalmic Review*.

In further search of the literature on the subject I have found no elaboration.

In order to understand the subject it is necessary to have a knowledge of the chemistry of both the aqueous and the crystalline lens.

Halliburton gives the composition of calves' aqueous as in Table 1.

TABLE 1.—COMPOSITION OF THE AQUEOUS

Water	986.87
Solids	13.13
Albumin	1.12
Extractives	4.21
Inorganic salts	7.70
Sodium chlorid	6.89

Thus it will be seen that it consists practically of normal saline solution with a trace of albumin and extractives.

The composition of the lens as given by Neumeister is set forth in Table 2.

TABLE 2.—COMPOSITION OF THE LENS

	Per cent.
Water	63.50
Solids	36.50
Albumins	35.00
Insoluble albuminoids	17.00
Crystalline (b)	11.00
Crystalline (a)	6.80
Albumin	0.20
Fats	.29
Lecithin	.23
Cholesterol	.22
Salts	.80

It will be seen that the lens contains 35 per cent. of protein, the highest protein percentage of any tissue of the body. Of this 35 per cent. protein, 17 per cent. is in the form of an insoluble albuminoid which is insoluble in normal saline or water.

It is a true albumin and constitutes most of the lens fibers, its amount increasing from without inward in accordance with the increasing age of the lens fibers. The remaining protein consists of a small amount of serum albumin and two vitellins which are soluble in dilute saline or in water.

If these analyses are correct, we have great difficulties in explaining the foregoing theories.

I can hardly agree with Parsons' statement that most of these fibers are carried off by the leukocytes. In the first place, if there is no infection there should not be many polymorphonuclear leukocytes about, as they occur chiefly in inflammatory troubles; and secondly, it would not explain the traumatic cataract with absorption in those cases of concussion without rupture of the capsule.

Fuchs seems to keep on the safe side by simply stating that the fibers become opaque and are finally absorbed, and by giving no explanation for the cause of the concussion cataract.

De Schweinitz's promulgation of Marcus Gunn's suggestion is, if the chemistry be correct, entirely untenable. To be sure the (a) and (b) crystalline bodies would be dissolved out either by water or the aqueous, but the insoluble albuminoid which makes up the greater portion of the protein, and which occurs chiefly in the lens fibers, is insoluble in either the water or the saline.

It is easily seen from a study of the chemistry of the lens why it becomes opaque either after injury with the entrance of aqueous or even from concussion. In case of rupture of the capsule and entrance of the aqueous there would occur an immediate precipitation of this insoluble albuminoid, while the remaining portions

1. Parsons: The Pathology of the Eye, 1904.
2. Schlösser: Experimental Studies on Traumatic Cataract, 1887.
3. Schirmer: Arch. f. Ophth., 1889.
4. Fuchs: Text-Book of Ophthalmology, 1908.
5. De Schweinitz: Diseases of the Eye, 1910.

would be soluble and could be carried off by the circulation of the aqueous. In cases of concussion without rupture of the capsule, there undoubtedly occurs some rupture of the lens fibers, and then this insoluble albuminoid is brought into contact with the lens lymph which acts in the same manner as the aqueous in precipitating the albuminoid.

But what is the factor which causes the solution of this albuminoid so that it can pass off in the circulation?

As we have known for several years that most of the organs and tissues of the body show autolytic activity under favorable conditions, we believe that this autolytic action also takes place in the lens substance after the rupture and breaking up of the lens fibers. There is also quite a possibility that there is some ferment in the aqueous which hastens this action. It is more probable, however, that the aqueous serves its chief purpose in carrying off the liquefied lens substance. Therefore, in order either to prove or to disprove our theories, the following experiments were carried out, using calves' eyes as the working material. They were enucleated at the abattoir and transported immediately to the laboratory and kept on ice until used, which was always within six hours after enucleation.

Nov. 1, 1909, I received thirty calves' eyes and drew off the aqueous with a sterile hypodermic needle. The lenses were removed and macerated, then diluted with normal saline solution, so as to make a dilute solution of lens albumin of about 1 per cent. This was agitated so as to make as nearly a homogeneous suspension of the insoluble portions as possible, and 5 c.c. were measured into three test-tubes. To the first were added 5 c.c. of the aqueous solution. To the second were added 5 c.c. of the boiled aqueous. To the third were added 5 c.c. of normal saline solution. These were stoppered tightly and placed in an incubator at 36 C. and allowed to remain for twenty-two days.

Chloroform and toluol were added to prevent bacterial action. They were then removed and the amount of soluble nitrogen was determined in each tube. They were precipitated by the action of heat and acetic acid, filtered and washed, and the amount of nitrogen determined in the filtrate by Kjeldahl's method, which consists of digesting with concentrated sulphuric acid and copper sulphate and distilling the remains, after rendering strongly alkaline, into a measured amount of tenth-normal sulphuric acid and titrating the excess of the acid.

By this procedure I obtained the results shown in Table 3.

TABLE 3

No. 1.—5 c.c. lens albumin 5 c.c. aqueous	= 2.1675 c.c. N/10 H ₂ SO ₄
No. 2.—5 c.c. lens albumin 5 c.c. boiled aqueous	= 1.9675 c.c. N/10 H ₂ SO ₄
No. 3.—5 c.c. lens albumin 5 c.c. normal salt	= 0.8175 c.c. N/10 H ₂ SO ₄

By this experiment it appears that the boiling of the aqueous has some inhibitory effect on its action on the lens albumin. It also appears that the aqueous either increases this process, or at least hastens it, because as will be seen, there is over twice as much soluble nitrogen in No. 1, which was combined with the unboiled aqueous, as in No. 3, in which there was only salt solution.

On account of lack of aqueous these experiments were not done with a control, so that the results are open to more criticism than they would otherwise be. All other experiments along similar lines with equal quantities of lens and aqueous were spoiled so that I lack confirmatory evidence, which I hope I shall be able to supply shortly.

Nov. 8, 1909, I started another series in the following manner:

Twenty calves' lenses were macerated with the accompanying aqueous, 15 c.c., and the whole made up to 500 c.c. with normal salt solution. Chloroform and toluol were added to prevent bacterial changes.

The preparation was then placed in a tightly stoppered flask and set in an incubator at 36 C., a measured amount being removed from time to time and the soluble nitrogen being determined by precipitating with heat and acetic acid and doing the Kjeldahl test on the filtrates as in the preceding experiment, with results given in Table 4.

TABLE 4.—VARIATIONS IN SOLUBLE NITROGEN AS DETERMINED BY HEAT AND ACETIC ACID

Date	N/10 H ₂ SO ₄ , c.c. Required by Soluble Nitrogen in 100 c.c.
11/ 8/09	4.8000
11/16/09	2.6750
11/23/09	3.5875
11/30/09	4.3375
12/ 7/09	3.8625
12/15/09	3.8375
1/ 5/10	4.0875
2/17/10	5.0625
3/17/10	5.8375
5/23/10	8.1175
10/25/10	8.9125

The autolytic material was now set at room temperature until October 25.

As will be seen by a glance at these figures there was a considerable lessening of the soluble nitrogen immediately after starting the process, after which, with practically one exception, a gradual rise until the quantity was nearly twice that of the original amount.

This initial decrease could be explained by the possibility of a reversible action of the ferment and a building up instead of a tearing down, as is seen in some other instances in physiologic chemistry, notably some of the fat ferments.

The influence of the small amount of aqueous in this experiment was, I believe, almost *nil*, as I will show in the following experiment a similar picture without the presence of any aqueous.

Nov. 18, 1909, twenty calves' lenses were macerated and made up to 500 c.c. with normal saline solution. Chloroform and toluol were added and placed in an incubator at 36 C., being placed in a tightly stoppered flask to prevent evaporation; 20 c.c. was removed from time to time and the precipitable protein removed by Abeles' method, which is as follows:

Fifty c.c. of absolute alcohol, 2.5 gm. zinc acetate, and 50 c.c. of the fluid are mixed and allowed to stand ten minutes and then filtered through a filter moistened with alcohol; the residue is rubbed up in a mortar several times and filtered; the zinc is precipitated with 20 per cent. sodium carbonate solution; this is filtered, acidulated with acetic acid, evaporated and a Kjeldahl test done on the filtrate.

This method is claimed to give more accurate results than the heat and acetic acid method. By the use of this method I obtained the following results:

TABLE 5.—VARIATIONS IN SOLUBLE NITROGEN BY ABELES' METHOD

Date	N/10 H ₂ SO ₄ , c.c. Required by Soluble Nitrogen in 100 c.c.
11/18/09	1.8375
11/22/09	2.5875
11/29/09	2.4625
12/ 6/09	2.5875
12/14/09	2.3275
1/ 3/10	2.4625
2/14/10	3.4625
3/17/10	4.4625
5/23/10	7.3675
10/20/10	7.3675

Set at room temperature until October 20.

In this and the preceding series, double estimations were made and when there was a variation the average of the results were taken.

It will be seen, as in the preceding experiment, that there was very little change for the first three months

and then there was such a pronounced change that one could hardly believe it to be accidental.

Here, as in the preceding series, there was little or no action while the material was at room temperature, from May to October, 1910.

Whether this was due to the slightly lowered temperature I cannot say. Neither can I say whether the increase in soluble nitrogen would have continued, as I had insufficient material to continue the experiment.

The tryptophan reaction was negative in both of these last experiments, on Dec. 11, 1910, showing that there was no very active ferment at work which would split up the amido-acids. Both preparations were tested for bacterial growth Dec. 12, 1910, by inoculating slant agar, with negative results.

If further investigation confirms my preliminary findings, then I think that it is safe to say that when the lens fibers are broken up they undergo autolytic changes. Whether this is increased by the action of some specific enzyme in the aqueous or whether it is due entirely to autolysis, it is difficult to say. If this be a ferment, then what is its source? Is it produced through the breaking up of the lens fibers, or is it derived from the circulating lymph? These questions I cannot answer.

These results coincide nicely with the facts as we know them. The lens of the young is much more easily liquefied and carried off, from the fact that there is less of the insoluble albuminoid present and the enzymes are all very active. In the aged, on the contrary, there is a much larger amount of the insoluble portion and all of the enzymes are relatively weak.

In closing I wish to thank Dr. E. L. Whitney for his many kind suggestions, and for the use of his private laboratory and apparatus at the Baltimore Medical College.

412 Cathedral Street.

TESTS OF THE VIRULENCE OF DIPHTHERIA BACILLI *

B. L. ARMS, M.D., AND E. MARION WADE, B.A.

Bacteriological Laboratory of the Boston Board of Health
BOSTON

Many times the laboratory worker is requested to test the virulence of diphtheria bacilli, especially in cases running for a long time, or in those occurring in institutions, and as we have had many interesting results from these tests we present some of them for consideration.

Our laboratory has always made virulence tests, on request either from the Board of Health or from the attending physician, the procedure being to isolate either by the streak-out method or surface seeding. For the former a series of eight or ten tubes is used, a fresh tube being inoculated from the incubated one; then, after sterilizing the needle, another from the freshly inoculated one, and so on through the series, taking from the first tube to the second, from the second to the third, etc., sterilizing the needle after inoculating each tube. In that way isolated colonies may be found on the later ones, as of course a smaller number of organisms is carried over each time. For the surface-seeding method five tubes are used, those being selected which contain a

fair amount of water of condensation. This is rubbed over the surface in order to insure an even distribution at the proper time. Inoculation is made directly into the water of condensation of the first tube, the needle is sterilized, and a loopful is carried into the water in the second tube; sterilization and inoculation being repeated throughout the series. The tubes are then laid down at the proper angle to cause the water to flow over the surface of the serum and allowed to remain for from two to five minutes, when they are taken up carefully and put into the incubator in the upright position. After incubation isolated colonies are "fished" and planted on serum and in sugar-free broth, and a microscopic examination is also made.

If there is no particular need for haste, or, in cases in which the organisms are very few, we sometimes make serum and microscopic examinations only, inoculating several colonies one above the other on a single serum tube, thereby making a great saving in the amount of media used; for many times we "fish" from twenty to forty colonies from a single series, especially from long-standing cases, the broths being made from the serum tubes after incubation.

These are incubated for forty-eight hours and pigs inoculated subcutaneously, using 0.5 per cent. of the body weight. The reaction may be local or general, or, as is usual in positive cases, both, and is generally present the following day, although a negative report is never given until forty-eight hours after the inoculation.

The virulence of morphologically typical diphtheria bacilli (Wesbrook's A C D) varies, not only in different cases but in different strains from the same case, from those absolutely non-virulent to those which cause death within thirty-six hours.

This was first brought to our notice in December, 1906, when a series of six pigs was inoculated, each with a different strain isolated from the same release culture.

In order to hasten the report one strain was inoculated at the end of the twenty-four hours and this proved virulent, the pig being so nearly dead on the second day that it was chloroformed.

The other five strains were all incubated for forty-eight hours and the difference in their virulence was very marked:

1. This was the pig previously mentioned.
2. This pig died on the eighth day, the reaction being local and general.
3. This pig died on the second day.
4. This pig showed a very slight local reaction on the second day, which increased slightly during the next week, but at no time was there a marked reaction. This pig was kept under observation for six weeks, complete recovery resulting.
5. This pig died on the ninth day.
6. In this instance there was a slight local reaction at the end of twenty-four hours which became more marked daily for some time and there was a slough surrounding the point of inoculation, the pig dying at the end of six weeks.

All of the above strains, grown fifteen hours on serum, were of Wesbrook's A A1 types. A rush of work prevented further tests at this time but an interesting problem had been presented.

Nov. 9, 1907, three pigs were inoculated with three strains isolated from a release culture, two dying within forty-eight hours and the other showing no symptoms; in order to rule out a possible difference in the resistance of the pigs we kept the cultures and eleven days later inoculated the surviving pig, and a new one to serve as a control, with the avirulent strain and at the

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