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A STUDY OF THE IMMUNOLOGICAL RESPONSE OF ROOSTERS TO THE INOCULATION OF LENS AND VITREOUS OF RABBITS' EYES.

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A STUDY OF THE IMMUNOLOGICAL RESPONSE OF ROOSTERS TO THE INOCULATION OF LENS AND VITREOUS OF RABBITS' EYES.

Since Uhlenhuth's[1] discovery of the organ specificity of lens, much interest has been shown in the problem by investigators with the result that in the last twenty years quite an extensive literature has accumulated bearing on the serological nature of lens and the other eye tissues.

Accordingly, in order to obtain the proper conception of the broader application of the problem and to interpret the meaning of any results obtained, a survey of this literature is necessary.

That the antigenic elements of the crystalline lens of the various species are organ specific, rather than species specific in character, is an accepted and well established fact. It was first brought to light in 1903 by Uhlenhuth, who demonstrated that an antiserum prepared by injecting rabbits with beef lens would give a strong positive precipitin reaction not only with an extract of beef lens but also with extracts of lens of

1. Uhlenhuth. Koch Festschrift, 1903, p. 49.

of other mammals, birds and amphibians. Uhlenhuth came upon this fact in the course of a series of immunological experiments in which he attempted to differentiate various proteins. For example, he succeeded in demonstrating:

1. A difference between chicken egg protein and that of chicken blood.
2. A difference in the proteins in the eggs of different fowls.
3. A difference in the proteins in a single egg.

His next step was to study eyes of different animals, pointing out that similar conditions existed in the egg and in the eye, namely the presence in one small chamber of two proteins, in the case of the eye, lens and vitreous. The lens protein, says Uhlenhuth, is related to mucin while the vitreous contains albumin and vitellin. Uhlenhuth's observation was that lens extracts give no reaction with serum antisera of homologous and heterologous bloods but that vitreous extracts do. His conclusion is that the two contain biologically different proteins. He states that by means of his anti-lens serum he is able to distinguish lens protein from vitreous and all other body proteins. Uhlenhuth did not produce an antiserum for vitreous.

By 1908, Kraus, Doerr and Sohma,[2] using anaphylactic

2. Kraus, Doerr and Sohma. Wien. Klin. Wchnschr. 1908, 21, p. 1084.

reactions as their indicator, had confirmed Uhlenhuth's discovery of the organ specificity of lens. In 1901 appeared a report of further work by Andrejew and Uhlenhuth[3] also attacking the problem from the standpoint of anaphylaxis, especially with regard to the lack of species specificity, their results checking with Uhlenhuth's earlier discovery. A repost of Peiffer and Mita's[4] study of these anaphylactic reactions was published in 1911 still confirming the work of the other investigators.

In 1910, Uhlenhuth and Haendel[5], Mita[6], and Krusius[7] approached the problem from a slightly different angle, and found that guinea pigs could be sensitized and intoxicated with guinea pig lens. Uhlenhuth and Haendel even went so far as to show that one lens of a guinea pig could be used to render the animal sensitive, while an extract of the other lens if injected after the proper interval of time, produced an anaphylactic shock. Roemer

3. Andrejew and Uhlenhuth. Arb. a.d.k. Gsndhtsamte., 1909, 30, p. 450.
4. Peiffer and Mita. Ztschr. f. Immunitatsf. 1911, 8, p. 358.
5. Uhlenhuth and Haendel. Ztschr. f. Immunitatsf. 1910, 4, p. 761.
6. Mita. Ztschr. f. Immunitatsf. 1910, 5, p. 297.
7. Krusius. Arch. f. Augenh. 1910, 67, p. 6.

and Gebb,[8] however, did not confirm these facts. The work of Andrejew,[9] and Kapsenburg,[10] indicates that certain species specific elements may be present.

In 1921, the first report of Hektoen's[11] investigations was published. Up to this time his work consisted mainly in confirming Uhlenhuth's original observation. A brief report is as follows: Rabbits were immunized with intravenous injections of 10% or 20% solutions by weight of beef, horse, sheep and swine lens. The antisera thus obtained were tested against solutions of beef, chicken, dog, guinea pig, horse, human, monkey, rabbit, swine, rat and sheep lens, aqueous, vitreous and serum. All the lenses reacted with all the lens antisera and none with the serum antisera. Aqueous and vitreous, in most instances, reacted in low dilutions with the lens antisera and frequently, though not always, with serum antisera. This, according to Hektoen, may be due to the mixture of the lens substance with the humors during removal but probably is not, for considerable care was taken. Considering these reactions with aqueous and vitreous, Hektoen points out that: "The pres-

8. Roemer and Gebb. Arch. f. Ophth. 1912, 31, p.367.
9. Andrejew. Ztschr. f. Immunitatsf. 1911, 8, p. 358.
10. Kapsenburg. Ztschr. f. Immunitatsf. 1912, 15, p. 518.
11. Hektoen. Jour. A.M.A. 1921, 77, p. 32.

ence of lens substance in the vitreous and aqueous humors raises interesting questions in regard to the relation of these humors to the lens. Is the lens substance in the humors derived from the lens or on the way to be incorporated into the lens? If the last part of the question is answered affirmatively, what is the source of the lens material?"

Hektoen then proceeded to analyze the antigenic elements of lens more closely. Using as antigens alpha and beta crystallins, the two globulins of the lens, which according to Hektoen[12] are described by Mörner, the former author and his co-worker, Shulof, prepared antisera which showed the two to be immunologically distinct so far as the precipitin reaction is concerned. Absorption experiments (e.g. mixing alpha precipitating serum and alpha crystallin and alpha precipitating serum and beta crystallin for a few minutes before a precipitin test is done) also showed that alpha and beta crystallin are distinct substances. Then by means of tests with lenses of different species and the anti-crystallin sera, it was found that lens in general contains alpha and beta crystallin. To these two globulins, suggests Hektoen, may the organ specificity of lens be attributed.

12. Hektoen. Discussion before Chicago Ophthalm. Soc. Nov. 10, 1922.

The reactions that Hektoen[13] obtained with cataractous lenses are also significant. His findings are these: The cataractous lens reacts as well with anti-lens sera as normal lens and produces as potent an anti-serum when injected. Using univalent sera (i.e. serum produced by immunizing an animal with an extract made from a single lens) no difference between normal and cataractous lenses could be determined by means of the precipitin reaction. Precipitin tests with anti-crystallin sera show cataractous lenses to contain less alpha than beta crystallin, a fact which is corroborated by chemical analysis.

Another valuable contribution of Hektoen's is the fact that a number of fetal human lenses of various stages of development, the youngest being at about the end of the second month, gave typical specific lens reactions but in much higher dilution with the anti-human cataractous sera than with anti-beef lens sera. Two of the fetal lenses reacted with human serum antiserum, the only in low dilutions apparently showing the presence of species specific proteins. In this connection the work

13. Hektoen. Jour. Infect. Dis. 31, No. 1, p. 72, July, 1922.

of Szily[14] should be mentioned. He reports the constant presence of species specific elements in the embryonal chick lens, a specificness which is apparently lost in the lens of the fully developed chick.

With respect to the anaphylactic reactions of lens and other eye tissues, the most recent and most extensive work is that of R. Kodama.[15] His results may be summarized as follows:

1. Extracts of all eye tissues are primarily toxic to the guinea pig. In degree of toxicity, the order is as follows: lens, uveal pigment, retina, uveal tissue, cornea and vitreous.

2. Taking into consideration the primary toxicity of those substances, the anaphylactic reaction indicates that that there is no absolute organ specificity for eye tissues.

3. There are marked differences in the range of the anaphylactic reactions of eye tissues. Lens is most limited. It is eye specific and nearly organ specific; that is, beef lens does not sensitize to beef serum but it does sensitize to other tissues of the eye. After lens, in order of their specificity as determined by anaphylactic reactions, come uvea, optic nerve, retina, cornea and vitreous. These all sensitize to beef serum and are

14. Szily. Klin. Monatsbl. f. Augenheilk, 12, p. 150, 1921.
15. Kodama. Jour. Infect. Dis. 30, p. 418, 1922.

species specific.

As Kodama states it "there are complicated group reactions within the eye tissues, as might be expected of tissues related embryologically, functionally and metabolically."

The reactions of uvea have been quite extensively studied. The first work in this field was done by Elschnig[16] in 1910, who undertook a series of experiments hoping to obtain the evidence necessary to explain sympathetic ophthalmia on the basis of an anaphylactic phenomenon. By inoculating sheep corpuscles, in one set of experiments, and in another, cholera vibrio extracts, into the anterior chamber of the eyes of rabbits and subsequently testing the bloodserum for the presence of specific antibodies, he established the fact that intraocular injections are capable of stimulating immunity reactions. His next problem was to determine whether or not uveal tissue possesses antigenic properties; if so, if such properties are potent in the homologous animal, to what part of the uveal tissue these properties are peculiar and if they are organ specific or species specific in

16. Elschnig. Arch. F. Ophth. 75, p. 459, 1910.
Ibid. 76, p. 509, 1910.
Ibid. 78, p. 549, 1911.
Ibid. 79, p. 428, 1911.

character. Elschnig used the complement fixation method for determining the presence of antibodies in sera prepared by inoculating rabbits with emulsions of heterologous and homologous uveal tissues. He established these facts:

1. That uvea does contain antigenic elements.
2. That antibodies are produced against homologous antigen.
3. That an immune serum produced with uvea of one species reacts also with uvea, but with no other tissue, of various other species and is organ specific.
4. That a serum immune to whole uvea tissue reacts not only with uvea but also with the serum of the animal whose uvea has been used for immunizing; that is, uvea apparently possesses also species specific elements. However, that such an antiserum does react with homologous serum may very probably be because a pure antigen, unmixed with blood, has not been obtainable. It must be remembered that a "pure antigen," that is a substance capable of stimulating the production of but one type of antibody, exists in theory alone so far as the body tissues are concerned. Lens approaches

homogeneity perhaps as closely as any part of the body, it having no blood supply, but even lens has been shown to be composed of at least two different proteins, alpha and beta crystallin. Until a tissue can be obtained absolutely free of blood, which of course is impossible except perhaps by culturing it in vitro, that tissue cannot be proven certainly to contain species specific elements in itself.

5. Finally, that uveal pigment is the active agent of uveal tissue, it being organ specific and showing no traces of specific specific elements.

Following Wlaschnig, numerous investigators have approached the problem of the serological reactions of uvea independently and from various angles, the result being a not exactly harmonious array of facts. Weichardt and Kummel[17], using complement fixation methods and the epiphanin reaction, the value of the latter being rather questionable, demonstrated strong but not absolute organ specific elements in beef uvea. Arisawa,[18] on the contrary, finds in sheep and calf uvea, using the entire uveal

17. Weichardt and Kummel. Munchen med. Wchnschr., 1911, 58, p. 1714.
18. Arisawa. XXXVIII Vers. d. Ophth. Gesells. Heidelberg, 1912, p. 253.

tissue, distinct species specific and weak organ specific elements, with complement fixation tests, while with precipitin tests the organ specific elements appear to be the stronger and the species specific the weaker. Szily,[19] studying the power of pure uveal pigment to produce anaphylaxis, obtained negative results. That is, injection of pigment into a supposedly sensitized animal produced apparently neither a general nor a local reaction. Nakamura, using complement fixation tests, finds uvea to be organ specific and not absolutely species specific.

Woods,[20] with the hope of interpreting sympathetic ophthalmia in terms of anaphylaxis, has conducted a rather extensive series of experiments and has done much to clear up this confusion of evidence. In brief, his work has been as follows: To quote Woods' statement of his problem: "The possibility or impossibility of this theory (the anaphylactic basis of sympathetic ophthalmia) hinges fundamentally upon the antigenic properties of uveal tissue, or, more exactly, upon the ability of uveal tissue to act as a foreign protein in the same species of animal, and

19. Szily. 17th Intern. Med. Congress. 1913, 9, p. 289.
20. Arch. Ophth. 45, p. 557, 1916.
Ibid. 46, p. 9.
Ibid. 46, p. 283, 1917.
Ibid. 46, p. 503, 1917.
Ibid. 47, p. 161, 1918.
Jour. A. M. A. 77, p. 1317, Oct. 1921.
Tr. Sect. Ophth. A.M.A. p. 133, 1917.

thus to produce sensitization and intoxication. Furthermore, uveal tissue, or the active constituent must be organ specific and lack species specificity". That uveal tissue possesses antigenic properties and that these properties are organ specific in character is established by the following experiments. Using as his indicator the local anaphylactic reaction of the eye, which consists of a marked contraction of the pupil and hemorrhages in the fundi, he found that dogs, sensitized with an intraperitoneal inoculation of uveal tissue, responded with a positive reaction when their eyes were perfused with normal difibrinated blood containing uveal tissue. Further, uveal tissue of any species was found to have an identical effect, in other words, it was organ specific.

In the course of these experiments certain things suggested that uveal pigment was the active agent, so his next experiments were designed to prove or disprove such indications. Elschnig previously claimed to have shown this to be true by means of complement fixation tests, but von Szily stated that his anaphylactic reactions did not confirm Elschnig's results. Woods saw a possibility of error in the method of preparation of the pigment antigen used by von Szily, and developed a more trustworthy method of his own. Von Szily, in the course of

the preparation of his antigen heated it to 80 degrees to coagulate the pigment. By such treatment, the pigment is rendered insoluble in all ordinary solvents, so it is not surprising that inoculations of such an antigen gave no anaphylactic reaction. Woods shows that, at autopsy, in animals inoculated intraperitoneally with the antigen prepared in this manner, the pigment can be demonstrated unabsorbed at the site of inoculation. This is evidently the explanation of von Szily's negative results.

Woods extracted the pigment from the uveal tissue by maceration in distilled water, filtered and precipitated with saturated ammonium sulphate. The precipitate which forms in the course of 24 hours consists theoretically of pigment, globulins and globin, the albumens having remained in solution. Repeated washings with distilled water remove all traces of ammonium sulphate and allow the globulins and globins to be redissolved. The precipitation of the pigment is, however, evidently an irreversible reaction, for it remains in suspension. In this form it is readily absorbed when inoculated. Thus Woods is dealing, theoretically at least, with a chemically pure substance. In experiments using local anaphylaxis as his indicator as before, he proves uveal pigment to be a

typical antigen, to be organ specific and not species specific. He farther shows that whole uvea possesses distinct species specific properties (as shown previously by Elschnig), thus proving that the pigment is the active principle in sympathetic ophthalmia. Woods emphasizes the importance of establishing the organ specificity of the pigment in the light of the following reasoning. "If the uveal pigment absorbed from the exciting eye produces hypersensitiveness of the second eye, intoxication in the second eye must be produced by uveal pigment alone, and not by other body protein. If this were not so, and other body protein could produce intoxication, the enucleation of the exciting eye, which is equivalent to the removal of the exciting pigment, would be of no value while other equally dangerous protein remained behind. If the pigment were not so lacking in species specificity, the blood serum, which is the "fluid extract of the organism", would theoretically produceintoxication. To establish a scientific basis for the anaphylactic theory, the pigment must be shown to be organ specific and not species specific. Thus, experimentally, Woods has established the facts necessary for the application of the anaphylactic theory. In addition to these experiments, in view of the conflicting results of other investi-

gators, Woods has repeated Elschnig's work with complement fixation reactions of uvea and uveal pigment antisera and believes his results to be confirmatory. Elschnig's statement was that whole uvea is species specific and that the pigment is not species specific but organ specific. Woods confirmed the first point absolutely, namely that whole uvea is species specific. With sera immunized against uveal pigment, a slight and variable fixation of complement was obtained with species specific antigens. However, since the reactions obtained were slight and not constant, and since the species specific reactions of the whole uvea were strong and constant, Woods is inclined to attribute them to impurities in the pigment antigen used in immunizing. Such an explanation is very probable, especially when the difficulty of obtaining chemically pure pigment is considered.

Finally, Woods has produced an experimental iridocyclitis in dogs by an intraocular sensitizing injection of uveal emulsion followed by an intoxicating intraperitoneal injection. The lesion is apparently a typical sympathetic ophthalmia.

A rather important contribution, bearing directly on the problem of sympathetic ophthalmia, is that of

Wissman[21] who has demonstrated uvea immune bodies in the sera of sympathetic ophthalmia cases by means of the precipitin reaction. Complement fixation reactions, however, gave negative results. Fuchs and Heller[22] were unable to confirm Wissman's work.

With regard to the other eye tissues, the work of Hess and Boemer[23], in which the retinal rods are demonstrated to be antigenic, Hektoen's observations that an anti-cornea serum reacts in precipitin tests with homologous serum, vitreous and aqueous and Rodema's work on anaphylaxis, mentioned above, represent the extent of our knowledge.

Recently some rather interesting clinical experiments and applications of the immunological properties of lens have been attempted. Verhoeff and Lemoine[24], considering the inflammatory reaction that frequently follows an injury to the lens, undertook to test their hypothesis that these inflammations could be explained on the basis

21. Wissman. Arch. f. Ophth. 80, p. 299, 1911.
22. Fuchs and Heller. Arch. f. Ophth. 88, p. 280, 1914.
23. Hess and Boemer. Arch. f. Augenheilk. 54, p. 13, 1906.
24. Verhoeff and Lemoine. Am. Jour. Ophth. 5, p. 700, 1922.

of congenital or acquired hypersensitiveness to lens protein. Formerly all such inflammations had been attributed to infection. Verhoeff's and Lemoine's[25] view was first suggested by Lagrange and Lacoste in 1911. Eight cases out of a series of 100 cataract cases (extractions) were reported by them with the suggestion that the inflammation was a result of irritation due to lens matter. Schirmer[26] observed cases of inflammation with sterile aqueous, but did not explain it on the basis of lens irritation. Straub[27] suggested that such intraocular inflammation be termed "Endophthalmia Phakogenetica" but did not consider the possibility of a hypersensitiveness to lens protein as the causative factor.

To ascertain whether individuals were hypersensitive to lens protein the ordinary technique of dermal or intradermal tests for sensitiveness to foreign protein were used. Ox, pig, or human lenses obtained at cataract operations were used as antigen. It was

25. Verhoeff and Lemoine. Am. Jour. Ophth. 5, p. 700, 1922.
26. Schirmer. Arch. of Ophth. 46, p. 167.
27. Straub. Am. Jour. Ophth. 5, p. 700, 1922.

attempted by these tests to determine the three following things:

1. Are patients who have intraocular inflammation apparently due to rupture of the lens capsule sensitive to lens? Each of twelve such cases examined gave a positive reaction.
2. What percent of individuals in general are hypersensitive to lens protein? Fifty tests were made, four of which were positive. Of these four, one was a case of interstitial keratitis, one a mature senile cataract, two immature cataracts operated by the intracapsular method.
3. Are patients who fail to show inflammation following injury to the lens, insensitive to lens protein? Thirty cases of perforation of the lens capsule, only two of which showed inflammatory reaction, were tested. The two cases with inflammation were the only ones to give a positive reaction.

Verhoeff and Lemoine also report a case, hypersensitive to lens protein, that was desensitized and successfully operated for immature cataract. As the patient gave a positive skin reaction to lens protein, eight intradermal inoculations of increasing strength were given

previous to operation. The operation was performed on the day after the last inoculation. As the lens matter was absorbed rather slowly, there being also moderate congestion, at the end of six weeks a second dermal test was made with a positive result. Whereupon intramuscular inoculation was begun again with definite improvement in absorption of lens and clearing up of the congestion. Weekly inoculations were continued until the condition was relieved. Verhoeff and Lemoine recommend that all cataract patients be tested and immunized if hypersensitive.

Davis[28] (1922) reports work done with regard to serum and vaccine treatment for the prevention and cure of cataract. Thirteen patients, inmates of the State Hospital for Insane, Central Islip, N.Y., were treated. Intravenous injections were first given, but anaphylactic reactions made the intradermal method necessary. The results of such treatment were that in two cases of mature cataract a great part of the cortical layers were absorbed. On the other hand, the opaque spicules in the immature cataracts were untouched.

The biologic aspect of the problem has been

28. Davis. Amer. Journ. Ophthalmol. 5, 745, 1922.

discussed by Guyer,[29] whose workhas been an invaluable contribution in this field. To quote from Guyer's paper on "Immune Sera and Certain Biological Problems" published in the American Naturalist in 1921, "---the phenomena which constitute the field of immunology, although today viewed mainly from the standpoint of infection and immunity, all have broader biological aspects. They must in the last analysis be but heightened or specialized reactions of the fundamental processes which underlie all life phenomena. They are but one of the many expressions of that delicately balanced stereochemical system we call protoplasm and are inextricably interwoven in the ebb and flow of metabolism, with such fundamental biologic processes as growth, reproduction, irritability and adaption." Guyer takes as the basis for his experiments the fact that specific cytotoxins have been found for such tissues as leucocytes, nerve tissue, spermatozoa and the crystalline lens, asking the question: If a serum can be produced that will single out a certain element of an adult organism, is it not possible that the representatives of this element in the germ will also be affected? This is a new

29. Guyer. Amer. Naturalist, 55, p. 97, 1921.
Ibid, 56, p. 80, 1922.
Ibid, 56, p. 116, 1922.
Jour. of Exper. Zool. 35, p. 207, 1922.
Trans. of an International Congress of Ophthalmol. Wash. D.C. 1922.

approach to the problem of acquired characters, of congenital anomalies and of the relations of mother and fetus.

Guyer's methods were these: Rabbit lens antisera were produced in roosters by injecting intravenously or intramuscularly 4 cc. of lens emulsion for four or five weekly treatments. The roosters were bled from seven to ten days after the last injection and the serum was then injected into pregnant rabbits with young advanced to the tenth day of pregnancy. Four to seven cc. of immune serum was injected at two or three day intervals for from ten days to two weeks. The total number of young subjected to this treatment was sixty-one. Of this number, four showed conspicuous eye defects, five had abnormal eyes and others manifested defects later in development. The commonest abnormality was opacity of the lens; other defects were: cleft iris, persistent hyaloid, bluish or silver color instead of the red of the albino eye, microphthalmia and almost complete disappearance of the eye ball. The defects were all attributable to early injury of the lens. In forty-eight controls, treated with unsensitized fowl serum, all eyes were normal. Once the anomaly was secured it was trans-

mitted to the eighth generation with none other than the original treatment. Moreover to rule out any possibility of additional antibody influence that the mother's serum might have on the fetus, the defect was transmitted through the male alone. Thus, responsibility for the defect must be assigned to the germ cell. It is significant, too, that the character is transmitted according to Mendel's law.

A defective male, mated with a normal female, produces apparently normal offspring, but a defective male mated with one of these offspring will produce defective offspring. Thus, the defective eye character acts as a typical Mendelian recessive.

Guyer's conclusion is, in the light of his experiments, that if antibodies from without, that is, those artificially introduced, can influence heredity, so can those normally produced within the body. In support of the hypothesis that antibodies can be produced against autologous tissues, Guyer cites the following evidence:

1. Römer, using complement fixation tests, found that the serum of adult humans may contain antibodies for their own lens proteins.
2. After inoculating male rabbits with their own

sperm, Guyer has demonstrated spermatotoxins in the blood. Further, the spermatozoa of these rabbits are weakened as shown in vitro by their lessened resistance to antiserum.

3. Blocking of one ductus deferens produces the death of the germinal epithelium of both testes. It is very logical to assume that the degenerating cells of the mechanically injured testicle have stimulated the production of antibodies that injure the other.

4. It is necessary to remove a severely injured eye to prevent "sympathetic degeneration" of the other. The work of Elschnig, Woods and others has proved experimentally that sympathetic ophthalmia is a manifestation of immune body production in response to the presence of degenerating, though autologous protein, namely uveal pigment.

5. In the late war, we frequently saw toxic effects resembling anaphylactic shock after extensive injuries of soft tissues. Thus Guyer's hypothesis may be summarized as follows: When all tissues are in normal physiological balance, no antibodies or modifying agents are developed. But in response to injury or undue stimulation serological changes do appear, to which

modifying influences the germ cells are exposed and by which they are undoubtedly affected both destructively and constructively. For example, the mole, living underground, suffered repeated injuries of the eyes. Consequently, regressive changes in that organ were the result. The mechanics of progressive changes is difficult to explain by application of this hypothesis or of any other, because of our lack of knowledge of the chemistry and physics of growth, hypertrophy and metaplasia. In answer to the question that is bound to arise, "Do you have to assume identical proteins in soma and in its germinal representative to make possible germinal variation as a result of immunite reactions?" Guyer says "Certainly not," and points to the facts of species and organ specificity and the "gradational reactions of immune sera according to the systemic relationship of animals" as giving a basis to his answer.

Guyer's work is revolutionary -- in its application to our ideas of heredity and suggests untold possibilities in the field of immunology. Provided his results and his explanation are substantiated, the old problem of the possibility of variation and evolution as a result of acquired characters, is finally answered.

But few references regarding the vitreous humor

are to be found in the literature. Uhlenhuth, as has already been mentioned, states that vitreous contains proteins distinct from those of the lens, as well as species specific elements. Possek[30] reported that neither organ nor species specific elements could be found in the vitreous. Kodama summarizes Trubin's[31] work by stating that that author obtained degenerative changes in the retina, pigment epithelium and choroid differing from the typical form of sympathetic ophthalmia with uveitis, by means of intra-ocular anaphylactic reactions, using beef and sheep vitreous as antigen. Kodama mentions the work of Nakamura[32], who using complement fixation tests, found vitreous to react with homologous vitreous and uvea and to react slightly with homologous lens. His reactions with homologous serum and heterologous vitreous were negative. Hektoen's results suggest that vitreous does contain traces of lens substance and that frequently, though not constantly, species specific elements are to be detected; that is, vitreous sometimes reacts with an homologous serum anti-serum. Besides these few observations, we have no serological evidence as to the nature

30. Possek. Klin. Monatsbl. f. Augenheilk, 1907, 45, p. 329.
31. Trubin. Graefe's Arch. f. Ophthalmol. 1915, 89, p. 227.
32. Nakamura. J. Komoto, Festschrift, 1913, p. 211.

of vitreous.

In view of our present knowledge of reactions of eye tissue and our lack of knowledge concerning those of the vitreous humor, it seemed advisable to undertake a study of the immunological properties of lens and vitreous. Especially with regard to its bearing on Guyer's experiments, was such an experiment deemed of value. It is a noteworthy fact that the anti-rabbit lens rooster sera used by Guyer in producing his eye defects in rabbits were in no way examined, so far as his reports how, as to their antibody content. Guyer assumes that specific cytolysins are responsible for the results he obtained but such cytolysins were not demonstrated. With this assumption of Guyer's in mind, consider the report of Hyde and Bailey[33] concerning their study of the production of hemolysins in the domestic fowl. Their results, which, however, have not been entirely confirmed by Menne[34], indicated that the fowl is incapable of producing hemolysins. Dr. Menne's work has shown that upon the repeated inoculation of sheep red blood cells, a typical antibody curve can be developed in roosters though

33. Hyde and Bailey. Amer. Jour. Hygiene. 2, p. 246, 1922.
34. Menne. Personal communication: Work in preparation for publication.

reactions were obtained with such anti-sera only in low dilutions. In the light of these facts, it is rather questionable whether it was specific cytotoxins for lens in Guyer's antisera that produced his eye changes. To my knowledge, there is no record in the literature of observations with regard to roosters as producers of lysins save that concerning hemolysins just mentioned, and a reference of Guyer's to positive in vitro tests for cytolysins in the serum of fowls previously inoculated with rabbit spermatozoa. Not that Guyer's theory that the embryonal eye is injured by specific immune bodies is necessarily erroneous, but a careful immunological analysis of such antisera as he used should be made.

In the present experiment, antisera were prepared by inoculating roosters with vitreous and extracts of lens of rabbits. Fresh rabbit eyes were obtained, the lenses removed intact by the extracapsular method and the vitreous separated with care from the rest of the eye. Occasionally bits of uveal tissue and retinal pigment were mixed with the vitreous but the presence of fragments of lens in quantity great enough to have disguised the antigenic properties of vitreous can be quite safely ruled out as the lens was removed unbroken and with considerable care.

Technic of Producing Anit-lens Serum.

Extracts of lens were prepared by shaking lens of a known weight in a known quantity of distilled water. After extracting for twelve hours, an equal quantity of double strength (1.8%) salt solution was added. The extract thus obtained was designated as of a certain per cent based on the original weight of the lens. This resulted in an approximately 20% lens in a 0.9% salt solution. This is, of course, not quantitatively correct as a large part of the lens is insoluble, but it furnished a standardization accurate enough for the purpose of immunizing. The anti-lens sera were produced by inoculating roosters intravenously at three day intervals with increasing doses, 2, 4, 8, 10, 12 cc. of a 5% extract. They were tested three, five and ten days after the last inoculation and bled when the precipitin titre was at a maximum.

Technic of Producing Anti-vitreous Serum.

Early attempts to stimulate antibody formation with intravenous injections of diluted vitreous over a long period of time gave negative results. The same rooster was then given intramuscularly pure vitreous which had been shaken vigorously to produce a fluid substance. Beginning with 2 cc. and increasing to 10 cc., a total quantity

of about 30 cc. was given in the course of five or six inoculations at three day intervals. With this technic, a potent antiserum was obtained.

It should be mentioned that after the rooster had been given a considerable number of intravenous injections of diluted vitreous, with apparently no antibody response, it developed symptoms of beri-beri. The diet on which all of the birds had been living was found to have been a very poor one and the symptoms were undoubtedly due to that cause. However, out of six roosters subjected to the same conditions of life and diet, the one treated with vitreous alone showed any ill effects. The question arises, is the astoundingly potent antiserum that, after the injections of concentrated vitreous, was obtained from this bird, to be associated in some way with this disturbance of metabolism in the course of the inoculation?

All antisera were preserved with a drop or two of chloroform as were also the antigens used for inoculation. Precipitins were demonstrated by the contact or ring test, the final reading being made at the end of one hour at room temperature. Since protein is precipitated to a certain extent by chloroform, fresh antigens

were used for all titrations.

The accompanying tables show best the nature of the antisera obtained. As shown in Table I, both the anti-lens and anti-vitreous sera reacted in very high dilutions with their specific antigens. Anti-vitreous serum reacted in equally high dilutions with lens while anti-lens serum reacted with vitreous in lower, but still in high enough dilution to consider the reaction a specific one. This seems to indicate that the proteins of lens and vitreous are identical. The startling fact shown by these titrations is the extreme delicacy of the anti-vitreous serum. While the anti-lens serum reacted in what would ordinarily be considered a very high dilution, 1-20,000, the former caused precipitation with lens antigen dilated to 1-6,000,000. This,moreover, is a very conservative figure. It is not the result of one titration, but of many done by several persons and with vitreous antigen obtained at different times. That vitreous is an extremely powerful antigen is indicated, though such a statement must be substantiated by inoculations of other animals. That the rooster is a particularly good producer of precipitins for lens and vitreous might also be concluded, but this also must be substantiated by further work. If these two assumptions prove to be correct, we have an interesting side light on the results of Guyer's experiments. It may be

that his lens extracts were contaminated with vitreous. If this were the case, his eye defects may have been due to injuries from specific vitreous antibodies. It is interesting and perhaps important to connect the embryological character of vitreous tissue with its apparent potent antigenic properties. Just what its significance may be can only be surmised in view of our present incomplete knowledge of what constitutes an antigen. When considering the fact that the antisera reacts apparently with vitreous only in lower dilutions, it must be remembered that the method of standardizing the antigen is quite inaccurate and further that pure vitreous contains many times less protein that lens.

Davis[35] gives these figures on the chemical composition of lens:

Protein.....	35.0%	(Hörner, as mentioned above has analyzed this protein as alpha and beta globulin.)
Fats, Lipins.....	0.75%	
Salts.....	0.75%	
Water.....	63.5%	

The only reference to be found with regard to the chemistry of aqueous and vitreous humors are in the Text Books of Anatomy. Cunningham gives for both humors

35. Davis. Amer. Jour. Ophthalmol. 5, p. 745, 1922.

approximately the same figures:

Water.....98.0%

NaCl..... 1.4%

Albumen.....Trace.

Rabbit aqueous also gave a positive reaction in low dilution, with both antisera. Here again the low protein content of the antigen must be considered in interpreting the reaction.

To ascertain whether any lens material is included with the vitreous when the lens is removed by the extracapsular method, two samples of vitreous were tested as shown in TableI. Antigen (1) was obtained by the ordinary method, while antigen (2) was obtained after the lens had been removed with its capsule intact. Each was tested against anti-vitreous serum. Antigen (1) gave a strong reaction in a dilution of 1-6,000,000, while antigen (2) gave only a faint precipitate in 1-5000,000. Evidently Antigen (1) was contaminated with fragments of lens. However, the anti-serum obtained with such an antigen was so powerful that it is impossible to consider that all the antibodies might have been produced in response to such minute quantities of contaminating lens material alone. Vitreous itself must be a true antigen. The apparent discrepancy between the figures given for these two titrations and the ordinary titre for this anti-vitreous serum against

vitreous is explained by the fact that the former are the exact readings obtained in a given experiment while the latter is an extremely conservative composite of the results of all the tests made.

That the proteins of lens and vitreous are identical as the inter-reactions of the two with their respective anti-sera indicates, is borne out by absorption tests, absorbing anti-lens and anti-vitreous sera each with lens extract and vitreous in dilutions of 1-20 for one hour, completely removes all antibodies for lens and vitreous that were previously present.

(See Table II)

The antisera were tested against rabbit serum and rabbit brain extract with negative results. (Table I). This indicates the absence of species specific elements.

Positive reactions were obtained with guinea pig lens and vitreous and anti-vitreous serum, reaffirming the organ specificity of vitreous. This is to be checked further by testing with vitreous of many species. With the lens antigen both antisera gave strong reactions in the last dilution tested (1-1280). The anti-vitreous serum reacted with guinea pig vitreous in as high dilutions as with rabbit vitreous.

The sera were titrated for agglutinins and

and lysins. The results are shown in Table III. Positive reactions occurred only in such low dilutions that they should be regarded as non-specific. Anti-lens serum #27 was prepared first and less care was taken in the preparation of the antigen. It reacts also in low dilutions with rabbit serum so its relatively high agglutinin titre for rabbit red blood cells is probably due to contamination of the antigen.

Conclusions.

1. Vitreous, like lens, is antigenic.
2. The anti-rabbit vitreous serum produced showed a higher titre for both lens and vitreous than did the anti-lens serum.
3. The fact that anti-vitreous serum produced reacts strongly with lens and anti-lens serum with vitreous indicates that in both tissues we are dealing with identical proteins. Absorption experiments bear this out.
4. Judging from the reactions of anti-rabbit vitreous serum with guinea pig vitreous and its failure to react with rabbit serum or brain extract, vitreous, like lens, is organ specific, and not species specific.

Table I.

Precipitin Reactions of the Anti-lens and Anti-vitreous Sera.

Antigen	Anti-lens serum	Anti-vitreous Serum.
Rabbit lens extract	20,480	6,000,000 plus
Rabbit vitreous	2,560	5,000 plus
Rabbit aqueous	32	32
Rabbit serum	0	0
Rabbit brain extract	0	0
Guinea pig serum	0	0
Guinea pig lens	1280 plus	1280 plus
Guinea pig vitreous	2560	5000+
Rabbit vitreous lens removed extracapsularly	2560	6,000,000 plus
Rabbit vitreous lens removed intracapsularly	1280	5,000,000 plus

Table II.

Absorption Experiments Showing the Identity of the Antigenic Elements of Lens and Vitreous.

Antigen	Anti-lens absorbed 1-20 lens for 1 hr.	Anti-lens absorbed 1-20 vitreous for 1 hr.	Anti-vitreous absorbed 1-20 lens for 1 hr.	Antivitreous absorbed 1-20 vitreous for 1 hr.
Lens	0	0	0	0
Vitreous	0	0	0	0

Table III.

Lysin, Agglutinin and Precipitin Titres to Check Species Specificity.

Antigen	Anti-lens serum #27	Anti-lens serum #33	Anti-vitreous serum #32
Rabbit red blood cells. Lysin titre.	6	6	6
Rabbit red blood cells. Agglutinin titre.	768	192	192
Sheep red blood cells Agglutinin titre	6	0	24
Rabbit serum Precipitin titre	16	0	0

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