

ANAPHYLAXIS PRODUCED BY EYE TISSUES

PRIMARY TOXICITY OF EYE TISSUES—ORGAN SPECIFICITY AND GROUP REACTIONS OF EYE TISSUES—ANAPHYLACTIC THEORY OF SYMPATHETIC OPHTHALMIA

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The biologic differentiation of animal, vegetable and bacterial species is established, but many questions in regard to organ specificity and group reactions of the proteins of animal tissues are still unsolved.

Uhlenhuth first showed the existence of organ specificity, differentiating the lens of beef¹ from the vitreous and other proteins of the same animal by the method of precipitation. This remarkable fact was confirmed by anaphylactic reactions by Kraus, Doerr and Sohma.² The lack of species specificity, here revealed, was studied further by Andrejew and Uhlenhuth³ by anaphylaxis and confirmed by Peiffer and Mita.⁴ Uhlenhuth and Haendel,⁵ Mita,⁶ and Krusius⁷ discovered also a new fact, namely that guinea-pigs may be sensitized and intoxicated by their own lens, but Roemer and Gebb⁸ did not confirm this. Though the serologic peculiarity and equality of lens protein through vertebrate animals is well recognized, the coexistence of some species specificity is indicated in the results of anaphylactic experiments, especially of Andrejew⁴ and Kapsenberg,⁹ while the precipitin reactions do not seem to reveal any species specificity in lens protein, as emphasized recently by Hektoen.¹⁰

When other tissues are considered, it is to be noted that Ranzi,¹¹ who worked with the liver, kidney and testicle of beef, horse and man as well as with malignant tumors, concluded that the anaphylactic state produced by organ extracts is not organ specific; the animals treated with one organ reacted with the same organ and other organs, also with the serum of the same species, giving no stronger reaction with the homologous organ than with other organs or the serum. Extracts of malignant tumors also sensitized to tumor and normal tissues and serum. However, Pfeiffer's¹² investigation on anaphylaxis with spermatozoa, kidney and blood led to the conclusion that injections with the pure proteins of those materials produced different anaphylactic states, which are to be regarded as organ specific, that is, there is a closer relation between

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¹ Koch Festschrift, 1903, p. 49.

² Wien. Klin. Wchnschr., 1908, 21, p. 1084.

³ Arb. a. d. k. Gsndtsamte., 1909, 30, p. 450.

⁴ Ztschr. f. Immunitätsf., 1911, 8, p. 358.

⁵ Ibid., 1910, 4, p. 61.

⁶ Ibid., 1910, 5, p. 297.

⁷ Arch. f. Augenh., 1910, 67, p. 6.

⁸ Arch. f. Ophth., 1912, 31, p. 367.

⁹ Ztschr. f. Immunitätsf., 1912, 15, p. 518.

¹⁰ Jour. Am. Med. Assn., 1921, 77, p. 32.

¹¹ Ztschr. f. Immunitätsf., 1909, 2, p. 12.

¹² Ibid., 1911, 8, p. 358.

spermatozoa and kidney than between spermatozoa and blood. Guerrini¹⁸ worked with the nucleoprotein of liver, spleen and serum of dog and horse and found that the anaphylactic reaction was organ specific, as only weak reactions could be obtained occasionally with the different organs of the same animal, but not between the same organs of different animals.

The eye tissues, especially uvea pigment, have interested ophthalmologists especially since Elschnig's¹⁴ pioneer work in which he applied the anaphylactic theory to sympathetic ophthalmia. His complement-binding experiments led to the conclusion that uvea antibody is not sharply organ specific and not species specific and that uvea pigment in its antigenic action is species specific. Weichardt and Kümmel¹⁵ obtained a strong but not absolute organ specificity with the serum of rabbits injected with beef uvea in experiments with the epiphanin reaction. Arisawa¹⁶ reports that immune serum for sheep and calf uvea reacted with homologous organs distinctly, but not at all or slightly with heterologous tissues, in short, a distinct species specificity and a weak organ specificity were observed in complement fixation tests, while in the precipitin tests the organ specificity appeared stronger and the species specificity weaker. Rados¹⁷ immunized rabbits with homologous uvea and cornea and demonstrated iso-antibodies which were neither organ nor species specific by the method of complement fixation. Szily,¹⁸ in his anaphylaxis experiments, found that his chemically pure uvea pigment did not give any reliable general anaphylaxis and gave no trace of local anaphylaxis by reinjection. Woods¹⁹ made perfusion experiments to study the local reaction and concluded that organ specificity was shown by uvea and its pigment in beef and dogs. Nakamura,²⁰ by complement fixation found that the uvea was organ specific and not absolutely species specific.

Uhlenhuth¹ observed that in precipitin tests the vitreous reacts with serum, contrary to the lens. Possek²¹ could find no organ or species specific properties in the vitreous. Trubin²² made intra-ocular anaphylactic reactions with beef and sheep vitreous as antigen and obtained certain degenerative changes of the retina, pigment epithelium and choroid, which differed from the typical form of sympathetic ophthalmia with plastic uveitis. Nakamura²⁰ found that in complement fixation vitreous reacts with homologous vitreous and uvea and slightly with homologous lens, but not with homologous serum and heterologous vitreous.

Concerning the other eye tissues, Hess and Roemer²³ found that the retinal rods have particular antigenic properties and produce antibodies that are lytic and agglutinating for rods, and recently Hektoen⁹ observed that beef cornea reacts with beef serum, vitreous and aqueous, but not with lens in precipitin reactions. Roemer and Gebb²⁴ made anaphylactic reactions with several eye tissues, but they withheld any conclusion for other tissues than lens, saying that the results were too inconstant and that more extended experiments were needed to determine the antigenic properties of the rest of those tissues.

¹⁸ Ibid., 1912, 14, p. 70.

¹⁴ Ibid., 1916, 20, p. 305.

¹⁵ München. med. Wchnschr., 1911, 58, p. 1714.

¹⁶ XXXVIII Vers. J. ophth. Gesells., Heidelberg, 1912, p. 253.

¹⁷ Ztschr. f. Immunitätsf., 1913, 19, p. 579.

¹⁸ Seventeenth Intern. Med. Congress, 1913, 9, p. 289.

¹⁹ Jour. Am. Med. Assn., 1921, 77, p. 1317.

²⁰ J. Komoto, Festschrift, 1919, p. 211.

²¹ Klin. Monatsbl. f. Augenheilk., 1907, 45, p. 329.

²² Graefe's Arch. f. Ophthalmol., 1915, 89, p. 227.

²³ Arch. f. Augenheilk., 1906, 54, p. 13.

²⁴ Graefe's Arch. f. Ophthal., 1912, 81, p. 367.

My earlier experience with the precipitin reactions of beef lens, cornea, vitreous, retina, uvea, optic nerve and serum as well as of horse and rabbit lens, which led me to the present work with anaphylaxis, indicated that strong antibeef-lens rabbit serum reacted distinctly with homologous and heterologous lens, and not at all with serum and that there were some quantitative differences between the reactions of lenses of different species. Such immune serums gave also weakly positive reactions with homologous cornea, vitreous and retina, especially at the acme of immunization; on the other hand, antibeef-cornea rabbit serum at the acme showed distinct reaction with cornea and weakly positive reactions with homologous vitreous, retina, optic nerve and serum.

From the review of the literature we see that the problems of the organ and species specificity of the tissues are far from solved and that the group reactions of tissues, particularly the relations between important eye tissues, have not been studied extensively. Moreover, the question of anaphylactic origin of sympathetic ophthalmia is still problematic. As stated before, V. Szily¹⁸ failed to substantiate Elschnig's conclusions, concluding from anaphylactic tests that the pigment is without antigenic properties; Woods,¹⁹ on the contrary, came to the conclusion that the uvea pigment has antigenic property, substantiating Elschnig's¹⁴ view that the pigment is responsible for the antigenic properties of uvea tissue. It seemed, therefore, of interest at this time to reinvestigate the antigenic properties of certain eye tissues.

GENERAL TECHNIC

The difference in the results obtained by various workers on organ specificity seems to be due partly to the admixture of blood constituents to the tissue proteins, partly to different methods of preparation of materials, determination of biologic reactions and estimation of the results obtained. The materials in this work were obtained from the fresh normal eyes of cattle killed by bleeding. The tissues were prepared aseptically, each tissue was isolated cautiously from the others and washed carefully with sterilized normal salt solution, and ground with quartz sand in mortar, after being cut in small pieces, then mixed with normal salt solution, and kept at 37 C. for 2 hours and shaken by machine for from 3 to 5 hours. All suspensions were filtered to secure fine emulsions. To obtain the uvea pigment pure, it was washed three times with salt solution and centrifugated carefully. For conservation a few drops of thymol solution were added. All materials were kept

in the icebox. The lens and vitreous of other animals (horse, rabbit, guinea-pig and mouse) were prepared by the same method; blood serum was obtained in the usual way. Fine emulsions were made of cornea, retina, uvea and optic nerve (intra-orbital part) in dilutions of 1 to 20; of lens in dilution of 1 to 100, and of uvea pigment in dilution of 1 to 1,000. The vitreous was used in full strength. The cornea, lens and vitreous body were absolutely free from blood and for the retina, uvea and optic nerve, it may be said that they were almost or nearly free from blood.

According to our knowledge, anaphylaxis is a most delicate and complicated biologic reaction which is open to sources of confusion and errors, if carried out without great caution. The action of surgical shock and the primary toxicity of the materials injected have not been considered carefully enough always. In the present work all possible care has been used to obtain reliable results, and I have estimated the results quantitatively on the basis of the fall of temperature by applying the Pfeiffer and Mita formulas: $\text{Shock units} = \frac{\text{temperature} \times \text{time}}{2}$. Thus, if in a given case the temperature falls 1.5 C. and returns to normal in 140 minutes, the calculation would be $\frac{15 \times 140}{2} = 1050$ shock units.

In fatal cases the formula used by Pfeiffer and Mita is as follows: $\text{Shock units} = 30,000 + (20,000 - \frac{\text{temperature} \times \text{time}}{2})$. Thus, if we assume that the temperature falls 4.5 C. and 60 minutes elapse before death, the calculation would be $30,000 + (20,000 - \frac{45 \times 60}{2}) = 48,650$ shock units. At the same time the general symptoms were studied carefully.

Guinea-pigs were sensitized by intraperitoneal injection, the intoxicating dose being given intravenously: After the animal was fixed on a board, the hair was cut, the skin sterilized and cut, the jugular vein then separated carefully and two threads placed under it; after the injection, these were tied firmly and the wound closed.

In our tables, the doses are given by weight in grams for the cornea, lens, retina, uvea and optic nerve, and in cubic centimeters for the vitreous body and serum.

PRELIMINARY EXPERIMENTS ON SURGICAL SHOCK

In many of the reports on anaphylactic experiments no attention seems to have been given to the influence of the operative procedure on results of the experiments. Some investigators have endeavored to account for this factor or have used some harmless route for the

intoxicating dose. While the subcutaneous or intraperitoneal injection seems to be practically harmless, the intravenous (jugular vein), intracardial or intracerebral injection may cause more or less shock by itself in a sensitive animal like the guinea-pig. This is especially important when one wishes to make a quantitative estimation of anaphylactic reactions. As stated, I chose the intraperitoneal injection for the sensitizing dose and the intrajugular route for the intoxicating injection, but first an effort was made to determine exactly the effect of such injections by themselves.

TABLE 1
EFFECTS OF INTRAJUGULAR AND INTRAPERITONEAL INJECTIONS OF SALT SOLUTION IN
GUINEA-PIGS

Temperature	Intrajugular Injections					Intraperitoneal Injections		
	Weight 430 gm. Amount Injected 2 c c	Weight 460 gm. Amount Injected 1 c c	Weight 265 gm. Amount Injected 0.5 c c	Weight 330 gm. Amount Injected 0.1 c c	Weight 225 gm. Amount Injected 0	Weight 435 gm. Amount Injected 10 c c	Weight 460 gm. Amount Injected 5 c c	Weight 25 gm. Amount Injected 1 c c
Before injection.....	40.0	39.8	39.1	38.5	38.8	39.0	39.0	39.3
Min. after injection								
10.....	39.0	39.0	37.6	36.7	38.1	38.7	38.8	39.3
20.....	39.0	39.5	37.8	36.9	38.4	38.7	38.7	39.3
30.....	39.1	39.6	38.0	37.1	38.8	38.6	38.7	39.3
60.....	39.5	39.6	39.1	38.3	39.1	38.8	39.0	39.6
90.....	40.0	39.5	39.7	38.9	39.2	39.0	39.8	39.9
120.....	40.5	39.7	39.6	39.3	39.5	39.0	40.2	40.1
150.....	40.7	39.9	40.1	39.4	39.7	39.5	40.1	39.7
180.....	41.1	40.0	40.1	38.9	39.8	40.3	40.1	39.6
210.....	41.0	40.1	40.4	39.1	39.6	40.2	40.0	39.6
240.....	40.9	39.8	40.3	39.2	39.7	40.1	39.6	39.6
270.....	40.8	39.9	40.5	39.0	40.0	40.0	39.6	39.5
300.....	40.9	39.8	40.3	39.0	39.9	39.6	39.7	39.6
330.....	40.8	39.9	40.6	39.2	39.8	39.4	39.6	39.5
360.....	40.8	39.9	40.6	39.1	39.9	39.2	39.6	39.4
390.....	40.9	39.8	40.6	39.1	39.9	39.2	39.4	39.4
420.....	40.8	39.8	40.4	40.4	39.9	39.1	39.3	39.3
Shock units.....	450	540	450	675	158	180	90	0
	Average 455					Average 90		

Normal guinea-pigs, weighing from 225 to 460 gm., were used. Five pigs were injected, respectively, into the jugular vein as described with 2.0, 1.0, 0.5, 0.1 and 0 c c of sterilized normal salt solution, and 3 pigs were injected with 10.0, 5.0, and 1.0 c c, respectively, of normal salt solution into the intraperitoneal cavity.

As the direct results of the intrajugular injection itself, the animals remained quiet for a while, the hair being ruffled, and sometimes trembling and increased tear secretion appeared. A short period of quiet, sometimes slight epiphora and a short apnea were noted after the intraperitoneal injection of a relatively large dose. The rectal temperature was tested in the same way as the anaphylactic temperature measurements are made, and I have estimated the shock value

by the Pfeiffer and Mita formula. The results are given in table 1, which shows that the intravenous injection itself caused the temperature to fall from 0.8 to 1.8 C., and that 60 to 150 minutes were needed to recover; thus the calculated shock values fluctuated from 150 to 675 units, the average value being 455 units. In the intraperitoneal injections, in which neither dissection nor bleeding occurs, the amount of fluid injected (1.0 to 10.0 c c) was apparently the only influence to account for the change of the temperature. The injection of 5.0 c c and 10 c c of normal salt solution caused the temperature to decrease 0.3 C. and 0.4 C., respectively, and the time for recovery was from 60 to 90 minutes, giving the shock values of 90 and 180 units; the injection of 1.0 c c of salt solution caused no fall in temperature.

PRELIMINARY EXPERIMENTS ON THE PRIMARY TOXICITY OF
EYE TISSUE EMULSIONS AND OF SERUM

In the literature on anaphylaxis the relation between the general primary toxicity and the anaphylactic reaction is a subject of much discussion. There are many articles concerning the primary toxicity of normal and pathologic tissues and of normal and immune serums, but comparatively few observations of this kind have been made on eye tissues, and on this account a preliminary study was made of their primary toxicity.

For this purpose normal healthy pigs weighing from 260 to 450 gm. were used, 4 for the cornea, 13 for the lens, 4 for the vitreous body, 3 for the retina, 3 for the uvea, 3 for uvea pigment, 3 for the optic nerve and 2 for serum. The cornea, retina, uvea and optic nerve were injected in 5% emulsions, the lens in 1%, and uvea pigment in 0.1% emulsions, the vitreous body and serum in full strength. The injections were made mostly into the jugular vein and partly intraperitoneally. The reactions were carefully studied by observation of the general symptoms and the temperature changes as well as by postmortem examination.

The general manifestations caused by the primary toxic action of the cornea, lens, vitreous body, retina, uvea and optic nerve were similar. Though some differences were noted, they seemed to be due to differences in quantity, in solubility and the individuality of the animals rather than to the inherent difference in the materials injected.

Generally speaking, guinea-pigs injected with one of the eye tissues mentioned showed the following phenomena: they became quiet, the hair rough, the body trembled, tears flowed, and there was more or less scratching, lapping and chewing; first slow, depressed, then

quickened and sometimes labored breathing was constant; relaxation of the muscles, paresis of the hind legs and drowsiness with narrowed lids often followed; the temperature first fell and later increased, but in the case of grave intoxication the latter did not occur; the lids and pupils were dilated and the eyeballs protruded when grave respiratory distress and convulsions came on; congestion of the ear lobes and of the conjunctivae and epiphora were common, but white discharge from the eyes was rare; convulsions were also rare; conjunctival hemorrhage was noted once. These are all principally similar to general protein toxicity and delayed anaphylaxis.

The anatomic lesions caused by the primary toxic action of eye tissues were similar, although some variations were noted, probably owing to the difference of concentration of the material injected and the individuality of the animal. Generally speaking, the lungs were often distended, with congestion usually and sometimes with petechiae, but pale lungs were found also, though rarely; the heart was often dilated, but not always; the myocardium was usually congested, the right side of the heart and vena cava usually holding soft coagula, while the left ventricle was empty; the abdominal organs usually showed congestion, but its grade varied; the intestines, especially the duodenum, the stomach, suprarenal, pancreas, liver, testicles and uterus usually showed marked congestion about in the order named; the skull, the pia, the subcutaneous tissue and muscles were also often found congested. These lesions were more or less similar to those of delayed anaphylactic death.

The temperature fall, fever reaction, absolute shock values (after deducting the surgical shock) and the outcome in each instance are shown in table 2, which shows that the cornea, lens, vitreous body, retina, uvea, uvea pigment and optic nerve of the beef and the lens of guinea-pigs may cause fatal intoxication in guinea-pigs due to primary toxicity, death occurring within from 5 to 73 hours. The minimal fatal dose per 100 gram body-weight for each eye tissue tested is as follows:

Beef cornea.....	0.322	Beef uvea.....	0.0357
Beef lens.....	0.000666	Beef uvea pigment.....	0.00329
Beef vitreous.....	0.938	Beef optic nerve.....	0.0313
Beef retina.....	0.0294		

Hence, the order of beef eye tissues according to primary toxicity is lens, uvea pigment, retina, optic nerve, uvea, cornea, and vitreous

body. Taking the vitreous body as the base, the scale of the primary toxicity of the tissues of the beef is as follows:

Crystalline lens.....	1408	Uvea	26
Uvea pigment.....	285	Cornea	3
Retina	32	Vitreous body.....	1
Optic nerve.....	30		

Heating at 56 C. for 1 hour 2 times reduced greatly the primary toxicity (uvea, optic nerve), and the fresh materials (rabbit lens and vitreous) gave higher fever reaction.

TABLE 2
PRIMARY TOXICITY OF EYE TISSUES AND SERUM OF BEEF AND OTHER ANIMALS

Guinea-Pigs, Number and Weight	Materials Injected		Place of Injection	Fall in Tempera- ture in 0.1 C.	Minutes Before Return of Temp. to Normal or Death Caused by Shock	Absolute or Net Shock	Results and Fever in Centigrade Degrees
	Kind	Amount					
1-285	Beef cornea	0.25	Jugular	21	150	1,120	Survived, 1.2
2-310	Beef cornea	0.1	Jugular	15	210	1,120	Survived, 1.9
3-285	Beef cornea	1.0	Peritoneum	12	180	900	Died in 5 hours
4-310	Beef cornea	1.0	Peritoneum	1	30	0	Died in 7 hours
5-385	Beef lens	0.1	Jugular	32	660	33,985	Died in 11 hours
6-260	Beef lens	0.05	Jugular	28	1,440	29,385	Died in 24 hours
7-350	Guinea-pig lens	0.02	Jugular	41	540	38,475	Died in 9 hours
8-500	Rabbit lens (fresh)	0.02	Jugular	21	600	5,845	Survived, 2.4
9-370	Beef lens	0.02	Jugular	33	300	4,495	Survived, 0.9
10-355	Mouse lens	0.02	Jugular	20	210	1,645	Survived, 0.6
11-345	Beef lens	0.01	Jugular	19	330	2,680	Died in 21 hours
12-360	Beef lens	0.01	Jugular	6	120	0	Died on third day
13-390	Beef lens	0.005	Jugular	17	150	825	Died in 21 hours
14-375	Beef lens	0.0025	Jugular	7	240	385	Died in 6 hours
15-310	Beef lens	0.0025	Jugular	9	150	220	Survived, 1.5
16-250	Beef lens	0.001	Jugular	24	60	265	Survived, 0.4
17-410	Beef lens	0.001	Jugular	10	60	0	Survived, 0.6
18-320	Beef vitreous	3.0	Jugular	14	240	1,230	Died in 24 hours
19-380	Rabbit vitreous (fresh)	3.0	Jugular	13	70	0	Survived, 1.4
20-410	Beef vitreous	2.0	Jugular	12	180	625	Survived, 0.6
21-405	Beef vitreous	2.0	Jugular	14	150	595	Survived, 0.5
22-285	Beef retina	0.2	Jugular	13	600	7,345	Survived, 0.5
23-340	Beef retina	0.1	Jugular	14	1,200	40,625	Died in 21 hours
24-450	Beef retina	0.1	Jugular	16	390	2,665	Survived, 0.2
25-280	Beef uvea	0.1	Jugular	32	390	43,305	Died in 6½ hours
26-400	Beef uvea (neated)	0.1	Jugular	29	180	4,765	Survived, 0.4
27-300	Beef uvea (heated)	0.05	Jugular	2	60	0	Survived, 0.0
28-390	Beef uvea pig- ment	0.05	Jugular	8	1,290	44,385	Died in 21½ hours
29-380	Beef uvea pig- ment	0.0125	Jugular	11	120	205	Died on third day
30-300	Beef uvea pig- ment	0.01	Jugular	5	90	0	Survived, 0.8
31-280	Beef opticus	0.1	Jugular	44	360	41,625	Died in 6 hours
32-320	Beef opticus (heated)	0.1	Jugular	26	180	1,865	Died on third day
33-300	Beef opticus (heated)	0.05	Jugular	3	60	0	Survived, 0.1
34-285	Beef serum	2.0	Jugular	22	180	1,525	Survived, 0.3
35-350	Horse serum (11 months old)	2.0	Jugular	12	67	0	Survived, 0.4

In mice and rabbits the intraperitoneal injection of suspensions of eye tissues may also cause severe symptoms of intoxication.

According to Wissmann,²⁵ human and beef eyes as a whole are toxic for guinea-pigs, but the lens, vitreous body, uveal tract and retina, injected separately, are harmless. The carrier of the toxic action of the eye seems to be the uvea and the retina which become active by the action of the lens and vitreous, which by themselves are not toxic; his results differ from mine and apparently they are not in accord with generally accepted principles. Dold and Ogata²⁶ studied the toxic action of watery extracts of rabbit tissues for rabbits, and they assert that the uvea plus the retina contain the most toxic substance, next the sclera and cornea, the lens and vitreous body not being toxic enough to cause death. Their results also differ from mine, but as their materials, methods of preparation and animals are also different, the results are not comparable.

ANAPHYLACTIC REACTION WITH EMULSIONS OF EYE TISSUES AND WITH SERUM

For this purpose normal healthy guinea-pigs weighing from 250 to 450 gm. were used in 7 different groups. The animals of each group were sensitized with one of the 7 materials prepared: the cornea, lens, vitreous body, retina, uvea, optic nerve, or beef serum. The sensitizing dose—0.05 gm. of cornea, retina, uvea, or optic nerve, 0.005 of lens, 1.0 c c of vitreous—was injected 3 times at short intervals into the peritoneal cavity; 1.0 c c of serum was injected once intraperitoneally. Between 18 and 25 days after the last injection, the intoxicating injection was made into the jugular vein; usually 0.01 or 0.02 gm. of lens, 0.1 gm. of cornea, retina, uvea and optic nerve, 2.0 or 3.0 c c of vitreous and of serum. The general symptoms, the change in temperature, and anatomic changes were carefully observed. The animals which escaped acute death were tested by subsequent injections as to refractoriness. The anaphylactic shock value was estimated by the fall in temperature, and the shock values due to surgical shock and primary toxicity were deducted for each case.

GENERAL CLINICAL PHENOMENA AND ANATOMIC CHANGES OF ANAPHYLAXIS CAUSED BY EYE TISSUES AND BEEF SERUM

The reactions of the animals which received reinjections of eye tissue, heterologous and homologous, and of serum after the sensitiza-

²⁵ Graefe's Arch. f. Ophth., 1912, 80, p. 437.

²⁶ Ztschr. f. Immunitätsf., 1912, 13, p. 667.

tion with one of our seven antigens may be classified as acute anaphylactic death, severe and moderate anaphylactic reactions and mild symptoms.

Acute Anaphylactic Death.—A few animals died instantaneously owing to respiratory stoppage in about 30 seconds, sometimes with a few agonal respirations. In these cases the lungs were usually distended typically, but sometimes there was no distention, which suggests a particularly severe intoxication of central origin; the heart usually beat strongly with dilatation and injection of the myocardium and contained fluid blood; the pia, cutis and abdominal organs sometimes showed some congestion. Many animals suffered typical acute anaphylactic death with great respiratory distress, violent convulsions and unconsciousness, which developed soon after injection, with defecation and micturition, death usually occurring in from 2 to 5 minutes. In these cases the lungs were greatly distended, emphysematous and mostly pale with more or less petechiae; the heart was dilated always, the walls congested and the blood fluid; the pia and abdominal organs usually showed more or less congestion, more marked when death was delayed; among the abdominal organs, the intestine, especially the duodenum, showed most intensive congestion, then the stomach, suprarenal, pancreas and sexual organs.

Severe Anaphylactic Reaction.—Many reinjected animals reacted with acute or delayed development of respiratory distress followed by convulsions, relaxation, paralysis, drowsiness, marked fall of temperature, congestion of the ear lobes and conjunctivae and an abundant white discharge from the eyes. Some died after several hours, up to 24 hours, and others survived. The postmortem examination gave less typical lung and heart findings, usually there was marked congestion of the pia, cutis, muscles and abdominal organs, generally resembling the changes caused by the primary toxicity of eye tissues.

Moderate Anaphylactic Reaction.—The animals became nervous, scratching and chewing, trembling, sometimes shaking the head, weeping and coughing, with roughening of the hair; the respiration first quickened, then slowed; partial relaxation and paresis, moderate fall in temperature and epiphora developed. These cases were not fatal directly.

Mild Symptoms.—The animals became a little nervous; slight respiratory changes and fever were observed; there were no fatal results.

TABLE 3
ANAPHYLACTIC REACTIONS OF GUINEA-PIGS TO TISSUES OF BEEF EYE AND OF BEEF SERUM

Sensitizing Injections (Intraperitoneal)	Weight	Reactions to Intoxicating Injections (Intrajugular)					Tests of Refractory State of Survivors						
		Days Since First Injection	Material Injected Intrajugularly	Fall in Temperature Normal in 0.1 C.	Minutes Before Return of Temperature to Normal or Death from Shock	Absolute or Net Shock	General Reaction	Hours Since Intoxicating Injection	Material of Third Injection (Intrajugular)	Fall in Temperature Normal in 0.1 C.	Minutes Before Return of Temperature to Normal or Death from Shock	Absolute or Net Shock	General Reaction
Beef cornea.... 0.15	360	21	Beef cornea... 0.1	7	2	48,418	Fulminant, death	1/2	Beef cornea... 0.1	11	2	48,414	Acute, death
	390	21	Beef lens.... 0.01	10	30	0	Slight.....	24	28	540	5,985	Moderate
	375	22	Beef vitreous 2.0	28	450	35,000	Severe, death					
	355	22	Beef retina.... 0.1	44	540	0	Moderate.....	4	12	1,440	7,065	Moderate
	370	22	Beef uvea.... 0.1	21	200	2,145	Moderate.....	21 1/2	25	330	3,045	Moderate
	290	23	Beef optics.... 0.1	23	390	47,993	Fulminant, death					
	270	23	Beef serum.... 2.0	27	2	48,704	Acute, death					
	335	23	Beef lens.... 0.005	21	2	0	Moderate.....	26	Beef lens..... 0.02	11	2	45,039	Acute, death
	350	21	Beef lens.... 0.0025	11	45	6,825	Moderate.....	26 1/2	Beef lens..... 0.01	17	90	0	0
	335	23	Beef cornea... 0.1	20	840	24,650	Severe, death	6	Rabbit lens.... 0.02	20	200	41,700	Severe, death
Beef vitreous... 3.0	360	25	Beef vitreous 3.0	18	2,700	1,650	Moderate.....					
	310	23	Beef vitreous 2.0	20	270	46,870	Acute, death					
	310	23	Beef retina.... 0.1	10	2	0	Moderate.....					
	380	25	Beef optics.... 0.1	10	120	0	Slight.....					
	410	21	Beef serum.... 2.0	12	120	0	Slight.....					
	450	21	Beef vitreous 3.0	23	4	48,274	Acute, death					
	430	23	Beef vitreous 2.0	42	440	38,710	Severe, death					
	370	22	Beef cornea... 0.1	27	4	48,371	Acute, death					
	385	23	Beef lens.... 0.02	6	3	45,041	Acute, death					
	410	22	Beef retina.... 0.1	9	2	46,871	Fulminant, death					
Beef retina..... 0.15	370	21	Beef uvea.... 0.1	44	540	32,900	Severe, death	28	Beef vitreous... 2.0	13	1,020	5,550	Moderate, death
	380	23	Beef optics.... 0.1	3	210	855	Moderate.....	3 1/2	Rab't vitreous 3.0	19	1,440	13,225	Slight
	390	21	Beef serum.... 2.0	27	470	2,520	Severe.....	8	Beef retina.... 0.125	17	2	46,863	Acute, death
	320	24	Beef retina.... 0.1	41	1 1/2	46,875	Fulminant, death					
	340	21	Beef cornea... 0.1	38	540	26,285	Severe, death					
	340	21	Beef lens.... 0.01	23	338	3,657	Moderate.....	8	Beef retina.... 0.125	16	2	46,844	Acute, death
	250	23	Beef vitreous 2.0	38	1,350	24,700	Moderate.....					
	385	23	Beef uvea.... 0.1	16	150	0	Slight.....	5	Beef retina.... 0.1	16	2	46,844	Acute, death
	370	23	Beef optics.... 0.1	36	1,620	26,820	Severe.....	29	Beef retina.... 0.1	17	2,430	17,535	Severe, death
	350	24	Beef serum.... 2.0	33	150	495	Moderate.....	5	Beef retina.... 0.1	19	2	46,860	Acute, death
Beef uvea..... 0.15	240	21	Beef uvea.... 0.1	46	1,440	27,000	Severe.....	26	Beef uvea.... 0.1	49	1,390	13,910	Slight
	290	21	Beef cornea... 0.1	37	420	6,195	Moderate.....	23	Beef uvea.... 0.1	41	1,800	31,680	Severe, death
	220	21	Beef lens.... 0.02	36	360	1,530	Moderate.....					
	235	19	Beef vitreous 2.0	17	5	48,007	Acute, death	24 1/2	Beef uvea.... 0.1	47	360	3,240	Severe, death
	480	19	Beef retina.... 0.1	13	300	0	Slight.....					
	450	19	Beef optics.... 0.1	40	15	47,360	Acute, death					
	300	20	Beef serum.... 2.0	25	5	47,957	Acute, death					
	270	21	Beef optics.... 0.1	22	3	47,957	Moderate.....	24	Beef optic N.... 0.1	47	35	46,887	Severe, death
	450	20	Beef cornea... 0.1	26	420	3,885	Moderate.....	24	Beef optic N.... 0.1	52	1,260	14,500	Severe, death
	300	20	Beef lens.... 0.02	15	180	0	Slight.....					
Beef optic nerve 0.15	460	21	Beef vitreous 2.0	18	5	48,875	Acute, death					
	370	21	Beef uvea.... 0.1	44	1,320	17,840	Severe, death					
	280	22	Beef uvea.... 0.1	43	570	32,525	Severe, death					
	380	22	Beef serum.... 2.0	21	5	47,967	Acute, death					
	290	22	Beef serum.... 2.0	20	2	48,000	Acute, death					
	330	24	Beef cornea... 0.1	18	240	585	Slight.....	4 1/2	Beef serum.... 2.0	14	5	47,985	Acute, death
	440	22	Beef lens.... 0.02	10	25	0	Slight.....	2 1/2	Beef serum.... 0.5	15	3	48,702	Acute, death
	435	24	Beef vitreous 2.0	13	5	48,917	Acute, death					
	435	28	Beef vitreous 2.0	40	900	30,950	Severe, death	23 1/2	Beef serum.... 2.0	11	4	47,098	Acute, death
	380	25	Beef retina.... 0.1	14	540	690	Moderate.....	1 1/2	Beef serum.... 2.0	20	3	47,990	Acute, death

Generally speaking, there is no principal difference between the anaphylactic reactions of different eye tissues and of serum, except that with materials from eye tissues there may be some irregularity of development of reactions as compared with serum anaphylaxis.

QUANTITATIVE ESTIMATION OF ANAPHYLACTIC REACTIONS BY THE TEMPERATURE FALL AND THE CLINICAL CLASSIFICATION

The rectal temperature of all animals which were used in anaphylactic experiments was measured exactly according to same method. The temperature was taken 3 times successively to begin with, then measurements were made three times at 10 minute intervals, then at 30 minute intervals. Thus the maximum temperature-fall in tenths of centigrade degrees and the time to recover or before death caused by shock in minutes were found, and the extent of shock was estimated by the formulae of Pfeiffer and Mita as explained already. Then the shock caused by the primary action of the eye tissue materials and the shock caused by the injection itself was deduced. The results are shown in table 3.

The tests of refractoriness or antianaphylaxis show that the animals reacted moderately or not at all on the third injection of the homologous antigen after the reinjection of homologous tissue or serum or tissue which is regarded as probably containing homologous anaphylactogen; on the contrary, the animals which received as second injection eye tissue, which is supposed to have little or no homologous antigen, died from typical anaphylactic shock on the third injection of homologous tissue or serum.

In the results we find rarely that the intoxicating injection of serum or of a different, but closely related, tissue gave a little more intensive reaction than the homologous tissue. This irregularity of reaction may be considered as due to differences of solubility and to the individuality of the animals.

THE ORGAN-SPECIFIC AND GROUP-REACTIONS OF EYE TISSUES AND SERUM

The results in the animals tested with emulsions of eye tissues and with serum have been recorded in the same terms and the results are summarized in table 4.

We see that there is no absolute organ-specificity in the narrow sense as commonly defined in any of eye tissues tested, although some of them may be organ-specific as concerns tissues outside the eye. Within the eye itself there are complicated interreactions or group

reactions of the embryologically, functionally and metabolically closely related tissues to a much greater degree than indicated in the previous investigations of these problems. In this respect my results may appear somewhat strange, but we know now that specificness does not depend on histologic structure but on chemical constitution.²⁸ According to my results, the general anaphylactogenic activities of the tissues of the beef eye and of beef serum as represented in table 5 indicate that the cornea and the vitreous body contain in greater or less degree all

TABLE 4
SUMMARY OF NET SHOCK VALUES OF ANAPHYLACTIC REACTIONS PRODUCED IN GUINEA-PIGS
BY EYE TISSUES AND SERUM OF BEEF

Sensitizing Antigens	Shock Values from Intoxicating Injections of						
	Cornea	Lens	Vitreous Body	Retina	Uvea	Optic Nerve	Serum
Cornea.....	48,418	0	5,250	35,000	0	2,145	47,993
Lens.....	6,825	48,704	13,120	46,870	0	0	0
Vitreous body.....	48,371	45,041	39,710	46,871	32,900	0	855
Retina.....	26,285	3,657	24,700	24,112	0	26,820	495
Uvea.....	6,195	1,530	48,907	0	27,900	47,360	47,957
Optic nerve.....	3,885	0	48,875	17,840	32,525	47,627	47,967
Serum.....	585	0	39,934	660	0	44,660	48,000

TABLE 5
THE GENERAL ANAPHYLACTIC EFFECTS OF TISSUES OF BEEF EYE AND OF BEEF SERUM

Sensitizing Injection	Intoxicating Injections						
	Cornea	Lens	Vitreous Body	Retina	Uvea	Opticus	Serum
Cornea emulsion.....	+++	0	++	+++	0	++	+++
Lens emulsion.....	++	+++	+++	+++	0	0	0
Vitreous.....	+++	+++	+++	+++	+++	0	+
Retina emulsion.....	+++	++	+++	+++	0	+++	+
Uvea emulsion.....	++	++	+++	0	+++	+++	+++
Opticus emulsion.....	++	0	+++	+++	+++	+++	+++
Serum.....	+	0	+++	+	0	+++	+++

+++ = strong reaction; ++ = moderate reaction; + = mild reaction; 0 = no reaction.

anaphylactogens of the eye tissues, while the lens and uvea contain the least number of associate anaphylactogens, the retina and optic nerve standing between.

Considering the anaphylactic theory of sympathetic ophthalmia on this basis, it is clear that uvea pigment is not the only possible source of the hypothetic active agent; the other elements of the uvea as well as constituents of other eye tissues may play a certain rôle, especially

²⁸ Abderhalden, E.: *Ztschr. f. Physiol. Chem.*, 1912, 81, p. 322. Wells and Osborne, *Jour. Infect. Dis.*, 1913, 12, p. 341.

in that form of sympathetic ophthalmia in which papilloretinitis is the prominent feature; here at least anaphylactogens of retinal and optic nerve origin might be the primary factors.

SUMMARY AND CONCLUSIONS

This work was undertaken to study the organ specificity and the group reactions of the eye tissues. As pure materials as possible were prepared. The cornea, lens, and vitreous body were used free from blood; the retina, uvea, uvea pigment and optic nerve were carefully washed as free from blood as possible.

The part played by surgical shock in the anaphylactic reaction was determined, and it was found that intrajugular injection itself causes a shock of from 150 to 675 units, according to the grade of injury to sensory nerves, the amount of bleeding and the individuality of the animals. The intraperitoneal injection causes a smaller degree of shock, varying from 0 to 180 units according to the amount of fluid injected.

The primary toxic effects of the tissues of the eye were determined for each tissue and they were found to be similar to the effects of protein toxicity in general and to delayed anaphylactic reactions.

Contrary to Wissmann, the lens, cornea, vitreous body, retina, uvea, uvea pigment and optic nerve of the beef and guinea-pig lens were found to be fatally toxic to guinea-pigs, mice and rabbits, death resulting in from 5 to 72 hours in guinea-pigs after intravenous or intraperitoneal injections. According to the minimal fatal dose the eye tissues stand in the following order according to primary toxicity: lens, uvea pigment, retina, optic nerve, uvea, cornea and vitreous body. Heating at 56 C. for one hour twice reduced the primary toxicity considerably, the fresh tissue materials producing a higher febrile reaction.

The specific anaphylactic reactions caused by the tissues of the beef eye were determined by studying the general symptoms, the anatomic changes, and the resulting refractory states; the shock values were estimated on the basis of fall in the temperature. Generally speaking, there is no essential difference between the anaphylactic reactions of the different eye tissues and of serum, but there may be some irregularity in the development of the reactions caused by ocular tissues, probably connected with questions of solubility and individuality.

The specific anaphylactic shock in each case was determined by deducing the effects of the primary toxicity of the material and quantity used and the shock produced by the injection itself. In this

way the organ-specific and group reactions of the eye tissues and serum were studied carefully. No absolute organ-specificity in the narrow sense as commonly defined since Uhlenhuth's discovery of the organ-specificity in precipitin reactions, was found in any of the eye tissues studied, not even the lens, though some may be specific for the eye. The results indicate that there are complicated group reactions within the eye tissues as might be expected of tissues that are related embryologically, functionally and metabolically. The facts at hand lead to the conclusion that the specific reacting units of anaphylactogens are not cells but chemical constituents of the tissues.

It appears that each eye tissue studied contains several antigens (anaphylactogens) in varying amounts, in addition to a specific or chief antigen peculiar to itself. The cornea and vitreous body contain in greater or less degree anaphylactogens that are common to all the different tissues of the eye. On the other hand, the lens and uvea contain the smallest number of associate anaphylactogens, the retina and optic nerve standing between.

If we apply these results to the anaphylactic theory of sympathetic ophthalmia, we must conclude that the active agent of that disease needs not to be uvea pigment alone as believed at present, as other elements in the uvea and in other eye tissues may play the same rôles as the uvea pigment is assumed to play. This enlargement of the anaphylactic theory of sympathetic ophthalmia readily takes in the form in which papilloretinitis develops, a process that the present pigment theory does not explain.