2. Grunberg-Manago, M., Ortiz, P. J., Ochoa, S., *Science,* 1955, v122, 907.

3. Kornberg, A., Lehman, I. R., Bessman, M. J., Simms, E. S., *Biochim. et biophys. acta,* 1956, v21, 197.

4. Nyc, J. F., Mitchell, H. K., *J. Am. Chem. Soc.,* 1947, v69, 1382.

5. Lieberman, I., Kornberg, A., *J. Biol. Chem.,* 1954, v207, 911.

6. Koritz, S. B., Cohen, P. P., *ibid.,* 1954, v209, 145.

7. Cohn, W. E., *J. Am. Chem. Soc.,* 1950, v72, 1471.

8. Hurlbert, R. B., Schmitz, H., Brumm, A. F., Potter, V. R., *J. Biol. Chem.,* 1954, v209, 23.

9. Chen, P. S., Jr., Torivara, T. Y., Warner, H., *Anal. Chem.,* 1956, v28, 1756.

10. Kornberg, A., in McElroy, W. D., Glass, B., *A Symposium on thhe Chemical Basis of Heredity,* Baltimore, 1957, 589.

11. Lieberman, I., *J. Am. Chem. Soc.,* 1955, v77, 2661.

12. Schmitz, H., Potter, V. R., Hurlbert, R. B., White, D. M., *Cancer Res.,* 1954, v14, 66.

13. Schmitz, H., *Biochem. Z.,* 1954, v325, 555.

14. Hurlbert, R. B., Potter, V. R., *J. Biol. Chem.,* 1954, v209, 1.

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Transfer of Experimental Panophthalmitis in Rats by Parabiosis.*† (24664)

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Transfer of experimental encephalomyelitis and glomerulonephritis by parabiosis was accomplished in rats(1,2). Transfer of glomerulonephritis has been demonstrated in both random bred and inbred strains of rats (3-5). The question arose, however, whether these transfer phenomena also held true for sensitization processes in other organs. In our experiments, allergic panophthalmitis has been produced by injecting rabbit-anti-rat-eye-serum into rats. The ophthalmitic animals were then joined with normal rats in parabiosis. Here, too, transfer of inflammation of the eye on the originally healthy partners has been achieved.

Methods. a) *Rabbit-anti-rat-eye-serum.* Frozen eyeballs of white rats of different breeds were triturated by the ballmill, diluted with saline to 20% suspension and injected 3 times a week for 2 months, and subsequently twice a week during 1 month, intraperitoneally into rabbits weighing 3-4 kg. Then the rabbits were bled from carotid arteries, blood collected under aseptic conditions, and antisera pooled and stored in 10 ml amounts in deep-freezer. b). *Cytotoxic panophthalmitis.* White rats of one strain (Stamm Zeilsheim) weighing 80-100 g, were injected with cytotoxic rabbit-anti-rat-eye-serum. Most animals were injected intravenously with single dose of 1-1, 8 ml of antiserum. Some rats also received that amount in 2 to 5 more daily injections. Starting 24 hours after first injection, the fundi of eyes were examined ophthalmoscopically every second day during experiment. The rats were sacrificed between 5th and 86th day after application of the antiserum. Whole eyes were fixed in 4% formalin, and sections of whole eye as well as of different tissues of eye were stained with H. + E. and PAS for microscopic examination. Sections of brain, heart, liver, spleen, and kidneys also were fixed in 4% formalin and stained with H. + E. Rats of the same strain, serving as controls, were untreated or injected with normal rabbit serum. Others were injected with 1 ml rabbit-anti-rat-kidney-serum intravenously. c) *Parabiosis.* As in former experiments(2) the parabiotic opera-

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TABLE I. Ophthalmoscopic and Histologic Signs of Panophthalmitis in 40 Rats Injected with Rabbit-Anti-Rat-Eye-Serum.

Group	No. of rats	Amt of antiserum	Occurrence of 1st ophthalm. findings (days after inj.)	Sacrifice (days after inj.)	Degree of ophthalmoscopic findings	Degree of histological findings
			Day			Degree
A	12	.7–1.8	2 × 1st 7 × 3rd 1 × 7th	2–21	2 × — 4 × + 1 × ++ 5 × +++	3 × 0 3 × 1st 2 × 2nd 2 × 3rd
B	12	1.0–3.0	1 × 1st 4 × 3rd 5 × 7th 1 × 9th	23–35	1 × — 2 × ++ 9 × +++	1 × 0 2 × 1st 4 × 2nd 5 × 3rd
C	10	1.0–2.0	3 × 1st 4 × 3rd 1 × 7th 1 × 9th 1 × 20th	36–59	2 × ++ 8 × +++	2 × 1st 5 × 2nd 3 × 3rd
D	6	1.5–2.0	2 × 1st 3 × 7th	69–86	1 × — 1 × + 4 × +++	1 × 0 2 × 1st 2 × 2nd 1 × 3rd

tion was performed by the method of Bunster and Meyer(6). By this technic, exchange of body fluids between parabiotic partners is based on capillary blood shunt established 3-4 days after operation. As in previous studies female rats have been used exclusively. Inbred Albino-Wistar rats (Stamm Koeln) and random bred white rats of one strain (Stamm Zeilsheim), in which ophthalmitic lesions have been recorded by ophthalmoscopic examination, were joined in parabiosis with healthy partners 8-43 days after the single or last injections of the cytotoxic rabbit-anti-serum. The animals were kept in parabiosis for 5-49 days and the same clinical and histologic examinations as described above for single animals were carried out in the parabionts. Parabiotic pairs without any injections were used as controls.

Results. Table I demonstrates the degree of clinical and pathological findings observed in 36 of 40 *single* rats, injected with rabbit antiserum. Clinical and histologic findings were divided in 4 degrees. The following symbols are used to indicate:

I. Ophthalmoscopic findings: — no pathologic findings; + hyperemia of fundus vessels, appearance of praecapillaries; ++ hyperemia of fundus vessels, appearance of praecapillaries, aneurysms, small bleedings from capillaries; +++ hyperemia of fundus vessels, appearance of praecapillaries, many aneurysms, perivascular sheath, large hemorrhages of retina, development of retroretinal exudate, sometimes followed by detachment of retina.

II. Histologic changes: 0 degree no pathologic changes. 1st degree hyperemia of iris, uvea, ciliary body, and retina. Vascularization of retina; 2nd degree hyperemia of iris, uvea, ciliary body, and retina. Vascularization of cornea, small infiltration of round and plasma cells in iris, cornea, and mostly in uvea; 3rd degree hyperemia of iris, uvea, ciliary body, and retina. Vascularization of cornea, compact infiltration of uvea, retina, and iris with round and plasma cells, occasionally eosinophiles. Edema of tissues of the eye, mostly of cornea. Retinal detachment, due to retroretinal exudation.

The first lesions were recorded by ophthalmoscopic examination within 24 hours after injection in 8 rats, within first 3 days in 15 others. They consisted of hyperemia of the retina and the chorioid tissue. Aneurysms and bleedings from capillaries of retina followed. Detachment of retina, due to retroretinal exudation, was observed in 11 animals. In one rat the lens became opaque, in 35 inflammatory changes were revealed by histo-

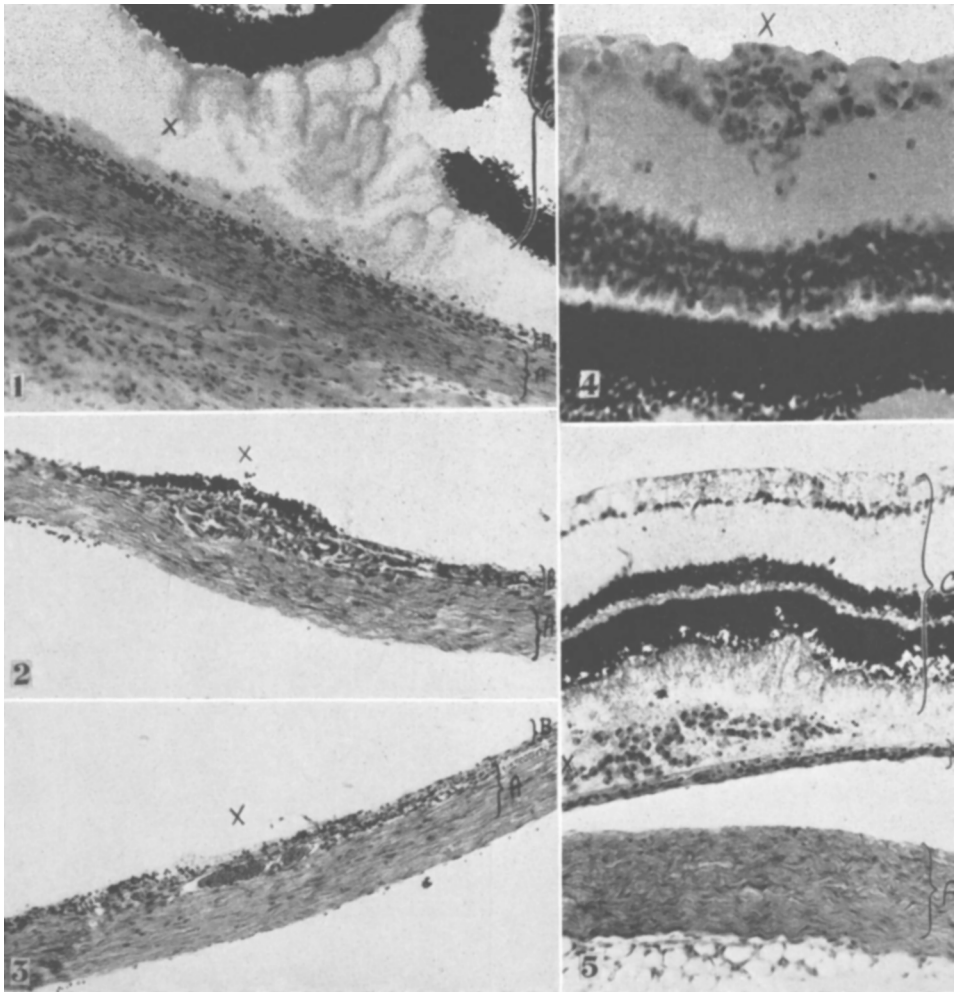

FIG. 1. Retroretinal exudation and secondary detachment. Intense exudation from densely infiltrated chorioidea resulted in retinal detachment. Rat eye stain: Hematox. + eosin. A = Sclera; B = Uvea; C = Retina; X = Exudate.

FIG. 2. Infiltration of round cells in chorioidea. At (X) dense concentration of small round cells. Chorioidea has taken on a cushion-like thickening due to edema. Rat eye stain: Hematox. + eosin. A = Sclera; B = Uvea.

FIG. 3. Perivascular infiltration of the chorioidea. (X) Infiltration of small round cells grouped around vessels in chorioidea. Rat eye stain: Hematox. + eosin. A = Sclera; B = Uvea.

FIG. 4. Perivascular infiltration of the retina. (X) Dense perivascular infiltration of round cells around a retinal vessel. Insignificant edema of retina. Rat eye stain: Hematox. + eosin.

FIG. 5. Infiltration of plasma cells. Massive immigration of plasmacellular elements from chorioidea into retroretinal space. At (X) densely concentrated plasma cells and retroretinal exudation. Secondary detachment in initial stage. Rat eye stain: Hematox. + cosin. A = Sclera; B = Uvea; C = Retina; X = Plasma cell infiltration.

logical examination. Fig. 1, section of eye, shows morphological lesions in one of these animals.

Twenty-eight rats, in which pathological findings were recorded by clinical examination after injection of antiserum ("primarily ophthalmitic animals") were joined in *parabiosis* with normal animals ("originally healthy partners") 8-43 days after injection. Three rats of the parabionts died within first 5 days after operation. In 21 of the remaining 25 pairs the ophthalmitis has been transferred on

TABLE II. Occurrence of Ophthalmitis (Recorded by Ophthalmoscope) in a Parabiotic Pair.

Days of exp.	Exp. procedures	Hyperemia of fundus vessels P	Hyperemia of fundus vessels O	Mikroaneurysms P	Mikroaneurysms O	Bleeding P	Bleeding O	Retroretinal exudate P	Retroretinal exudate O	Detachment of retina P	Detachment of retina O
1	Inj. of 2.0										
3	serum	+									
5		+		+							
7		+		+							
9		+		+							
11	Parabiosis	+		+							
13		+		+		+					
15		+	+	+		+					
17		+	+	+		+					
19		+	+	+	+	+					
21		+	+	+	+	+		+			
23		+	+	+	+	+	+	+			
25		+	+	+	+	+	+	+			
27		+	+	+	+	+	+	+			
29		+	+	+	+	+	+	+		+	
31		+	+	+	+	+	+	+		+	
33	Sacrifice	+	+	+	+	+	+	+	+	+	+
35		+	+	+	+	+	+	+	+	+	+

Histologic findings:

P = Primarily ophthalmitic animal—Compact infiltration of uvea with round cells, plasma cells and eosinophiles. Retroretinal exudate, detachment of the retina. General hyperemia respectively edema of the tissues.

O = Originally healthy partner—General hyperemia of uvea and retina. Infiltration of round cells in uvea and iris. Vessels in cornea. Detachment of retina.

the originally healthy partners.

Table II exemplifies in one pair time relations between injection of rabbit antiserum into the first animal, development of ophthalmitic lesions, recorded by ophthalmoscopic examination, parabiotic union and, eventually, occurrence of ophthalmitis in the originally healthy animal together with final histologic findings.

Table III summarizes the same relations in all parabiotic pairs joined in parabiosis at various intervals after injecting rabbit antiserum into the primarily ophthalmitic animal. Without relation to these different intervals, panophthalmitis occurred in originally healthy parabionts between 4th and 12th day after parabiotic union, and the 11th and 50th day after injection of antiserum into primarily ophthalmitic partners respectively. Histologic findings in the originally healthy parabionts were similar to those recorded in single animals injected with rabbit antiserum, and injected animals of the parabiotic group: Infiltration of round cells in uvea with edema in tissues (Fig. 2). Perivascular infiltration of round cells in chorioidea (Fig. 3), and in retina (Fig. 4), infiltration of plasmacells from uvea into the retroretinal space and beginning of retinal detachment (Fig. 5). No pathological findings were observed in sections of brain, heart, spleen, liver, and kidneys in either of these groups. Only occasionally small areas with infiltration of round cells were present in the injected animals in interstitial tissues of the liver. On the other hand, lesions of the eye were completely lacking in all untreated single animals, in 10 rats injected with normal rabbit serum and in 10 parabiotic controls. No signs of ophthalmitis were found in 10 rats injected with anti-rat-kidney-serum of rabbits. In several animals of this group, however, the well known signs of hypertensive diseases have been recorded in eyegrounds after development of albuminuria and hypertension.

Discussion. The experiments show that allergic panophthalmitis can be easily produced by injecting rabbit-anti-rat-eye-antibodies into rats. These observations are completely in accord with the findings of Golowin(7), who produced iridocyclitis by injection of antibodies against ciliary body ("cyclotoxins").

TABLE III. Incidence of Panophthalmitis in Parabiosis.

Group	Rat No.	Interval between inj. and institution of parabiosis (days)	Occurrence of first ophthalmoscopic changes in orig. healthy rats (days after parabiosis)	Degree of ophthalm. findings P	Degree of ophthalm. findings O	Degree of hist. findings P	Degree of hist. findings O	Day of sacrifice After inj.	Day of sacrifice After parabiosis
E	41	8	6	++	+++	1	3	26	19
	42	8	4	+++	+++	3	3	13	6
	43	8	—	+	0	1	0	14	7
	44	8	—	+	0	1	0	14	7
	45	8	6	+	++	1	2	20	13
	46	8	4	+++	+++	3	2	56	49
	47	8	6	+++	+++	2	1	23	16
	48	9	—	+	0	1	0	9	1
	49	9	8	+++	++	3	3	59	51
	50	9	8	+++	+++	2	3	21	13
	51	10	5	+++	++	1	2	32	23
	52	10	7	++	+++	2	3	40	31
	53	10	6	+++	+++	3	3	35	26
F	54	14	—	+++	0	1	0	31	18
	55	17	6	+++	++	1	0	31	18
	56	17	6	+++	++	2	2	25	9
	57	17	9	+++	+++	1	3	35	19
	58	17	12	+++	+++	3	3	37	21
G	59	26	—	+++	0	2	0	29	4
	60	26	—	+++	0	2	2	30	5
	61	26	—	+++	0	3	0	31	6
H	62	37	5	+++	++	2	1	70	34
	63	37	5	++	+++	2	3	48	12
	64	37	5	+++	++	2	2	48	12
	65	43	8	+++	++	2	2	68	26
	66	43	4	++	++	2	2	48	6
	67	43	7	++	+	2	1	55	13
	68	43	5	++	+++	2	3	49	7

Explanation: P = Primarily ophthalmitic animal; O = Originally healthy partner.

Lesions initiated by our antiserum occurred in all parts of the eye because whole eyes were used for immunization. Their strict limitation to tissues of the eye, however, implies an extraordinary specificity of rabbit tissue antibodies, which is rare in cytotoxins. Rapid development of panophthalmitis after injection suggests the same immediate type of passive and reverse anaphylaxis as has been found in experimental glomerulonephritis of the rat after injection of rabbit nephrotoxins (8,9). Our experiments show furthermore that experimental panophthalmitis was transferred in 21 of 25 pairs from primarily ophthalmitic rats to originally healthy partners by parabiosis. Lesions of the eye occurred in the originally healthy animals between 4th and 12th day after parabiosis, irrespective of whether an interval of 8-10 or 37-43 days had elapsed between injection of cytotoxic antiserum into primarily healthy animal and institution of parabiosis. Thus, in the latter group, the first signs of panophthalmitis were recorded in primarily healthy animals between 41st and 50th day after injecting rabbit antiserum into the partner (group H, Table III). Similar observations have been made in our previous experiments in which after intervals of 30-45 days (and recently after 71 days) between injection of rabbit nephrotoxins and parabiotic union, glomerulonephritis has been transferred to originally healthy rats(4,5). The question, therefore, arises whether or not panophthalmitis in originally healthy parabionts was produced by the heterologous rabbit-anti-eye antibodies injected into the primarily ophthalmitic animal. Experiments are in progress to elucidate the immunological

mechanism of this transfer phenomenon, *i.e.*, whether sensitization of the eye of the originally healthy partner is due to surviving injected heterologous antibody or to some other not yet clearly determined secondary tissue damaging factor.

While the transferred ophthalmitis generally was less pronounced than in the injected animals, the characteristics of inflammatory lesions of the eye were the same. In both, all of parts of eye tissues were affected, and the delayed tuberculin-type of hypersensitivity reaction was indicated by lymphoid and plasma cells in the inflamed areas(10). However, as this type of anaphylaxis has been produced in injected animals by serum antibodies, it cannot be decided whether transfer of ophthalmitis was produced by serum antibodies or antibody containing white cells of the primarily ophthalmitic animal.

Discussions of clinical implications of our findings are beyond the scope of this paper. However, certain similarities are obvious between panophthalmitis produced by injection of anti-eye-antibodies and transferred by means of parabiosis, and the human disease called sympathetic ophthalmia. With respect to whether nervous factors are involved in this disorder, it seems justified to stress that nervous elements cannot be implicated in our experiments. Certainly, this experimental panophthalmitis has been produced only by humoral factors. This recalls the old concept of autoallergic processes in sympathetic ophthalmia(11), production of inflammation of the lens by sensitization with homologous lens material(12) and the newer therapeutic approaches with steroid hormones in this disease(13).

Summary. 1. Experimental panophthalmitis has been produced in 36 of 40 rats injected with antiserum of rabbits against rat eye tissue within 24 hours after injection. Clinical and histological features of eye lesions are described. 2. Twenty-eight rats in which pathological findings were recorded by ophthalmoscopic examination after application of the antiserum ("primarily ophthalmitic animals") were joined in parabiosis with normal animals ("originally healthy partners") 8-43 days after injection. Three rats of the parabionts died after operation. In 21 of the remaining 25 pairs, transfer of panophthalmitis on originally healthy partners occurred between 4th and 12th day after parabiosis and the 11th and 50th day after injection of antiserum into primarily ophthalmitic animals respectively. Ophthalmoscopic and histological findings of transferred ophthalmitis were similar to those induced by injection. 3. The intervals between injection of antiserum into primarily ophthalmitic rat, parabiosis and occurrence of ophthalmitis in the originally healthy partners lead one to ask whether the panophthalmitis in the originally healthy parabionts is due to surviving injected heterologous antibodies or to some other not yet determined secondary tissue damaging factor.

1. Lipton, M. M., Freund, J., *J. Immunol.* 1953, v71, 380.

2. Pfeiffer, E. F., Schöffling, K., Sandritter, W., Schröder, J., Steigerwald, H., Wolf, L., *Z. exp. Med.*, 1954, v124, 471.

3. Pfeiffer, E. F., *Zusammenkunft Dtsch. Ophthalm, Ges.*, Heidelberg, 1957, v61, 90.

4. ———, Ninth Annual Conf. on Nephrotic Syndromes, Bethesda, Md.

5. ———, Henry Ford Hosp. Intern. Symp. on Mechanisms of Hypersensitivity, Detroit, 1958.

6. Bunster, E., Meyer, R. K., *Anat. Rec.* 1933, v57, 339.

7. Gclowin, S. (quoted in Miescher, A. P., *Immunpathologie,* Thieme, Stuttgart, 1957, p553: Russky Wratsch, Nr. 22, 29 May 1904.

8. Pfeiffer, E. F., Schöfflng, K., Bruch, H. E., Spielmann, W., *Verh. Dtsch. Ges. Inn. Med.* 1953, 453; *Z. exp. Med.* 1954, v122, 446.

9. Pressman, D., Korngold, L., Heyman, W., *Arch. Path.*, 1953, v55, 347.

10. Chase, M. W., *J. Bact.*, 1946, v51, 643.

11. Golowin, S.: (quoted in A. a. P. Miescher etc.) *Klin. Mb. Augenheilk.* 1909, v47, 150; Woods, A. C., *Trans. Ophthalm. Soc. United Kingdom,* 1925, v45, 208.

12. Burky, E. L., *Arch. Ophthalm.* 1934, v12, 536.

13. Moulinho, H., *Bull. Soc. Ophthalm. France,* 1950, 652.

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