

the whole is placed in the large press under an average of 16,000 pounds per square inch of pressure. When small pieces of tissue are extracted, about 15% to 20% by weight is recovered as fluid. The juice thus obtained contains considerable fat and is therefore allowed to stand in the icebox overnight. The following morning it is centrifuged in the cold centrifuge† and the clear fluid which lies underneath the fat is removed with a capillary pipette. This extracted fluid is suitable for carrying out either precipitin or agglutinin determinations. When it is to be used for precipitin determinations, repeated centrifuging in the cold centrifuge is usually advisable. Since these organ extracts sometimes precipitate spontaneously when incubated at 37°C., all tests are put in the icebox immediately and are read in 24 hours and again in 48 hours, according to the method of Heidelberger, Kendall and Soo Hoo.¹ The end titers obtained in the icebox are the same as those obtained by a preliminary incubation at 37°C. followed by icebox treatment.

The pressure exerted by this method of extraction is apparently sufficient to break up body cells, for stained films of the residue of dry filter paper and tissue have failed to show any intact cells.

The agglutinin titer of tissue juice obtained by this method has been compared with that obtained by the method of Cary² of grinding with sand and extracting with equal parts of physiological saline and glycerine. The titers obtained by the pressure extraction are equal to or higher than those obtained by the method of Cary.

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Local Organ Hypersensitiveness. VII. Demonstration of Agglutinins in Tissues of the Rabbit Eye Following Immunization with Eb. Typhi Vaccine.

BEATRICE CARRIER SEEGAL AND DAVID SEEGAL.

From the Departments of Bacteriology and Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York.

The injection of a foreign protein into the anterior chamber of the rabbit's eye results in a local sensitization of this organ. Weeks

† Manufactured by the International Equipment Company, Boston, Mass.

¹ Heidelberger, M., Kendall, F., and Soo Hoo, C. M., *J. Exp. Med.*, 1933, **58**, 137.

² Cary, W. E., *J. Med. Research*, 1922, **43**, 399.

or months later when the eye has returned to a normal appearance the intravenous injection of the same protein produces a marked inflammation of the iris and conjunctiva. The opposite, control eye shows no reaction or only a slight hyperemia.¹ This local hypersensitivity has the characteristics of an antigen-antibody reaction.^{1, 2} The presence of antibodies in the tissues of the hypersensitive eye was not demonstrated, however, by the technique previously employed.³

This paper reports further studies on the antibody content of the juices extracted from the tissues of the injected eye. By an alteration in the technique of extracting the tissues and the utilization of a particulate antigen which stimulates the production of agglutinins it has been possible to demonstrate the concentration of antibody in the tissues of an eye injected with typhoid vaccine.

A series of 12 adult albino rabbits were injected into the anterior chamber of the right eye with 0.1 or 0.15 cc. of New York City Board of Health typhoid vaccine which contained approximately 3 billion organisms per cc. The injection was given under cocaine anesthesia by the method already described.¹ The animals were sacrificed 4 to 102 days later by bleeding from the heart. Sixty to 120 cc. of blood was obtained. The anterior chamber fluids were removed and the palpebral and scleral conjunctiva together with the tissues from the anterior two-thirds of the eyeball from each eye were dissected out. The lens was expelled and the excess aqueous and vitreous humors were blotted off. The tissue juice from these fragments of tissues, consisting of conjunctiva, iris, sclera and cornea, was extracted with pressure by a method previously described.*³ The fluids so obtained were tested for their agglutinin titer and the titers compared with those obtained in the serum and the aqueous humors. A series of 11 control animals was similarly tested. The treatment of the controls is indicated in Table I.

The results of the experiments are summarized in Table I. In those animals injected with the typhoid vaccine in the anterior chamber of the eye, agglutinins appeared first in the serum. During the first month following the injection of the vaccine the serum

¹ Seegal, D., and Seegal, B. C., *J. Exp. Med.*, 1931, **54**, 249.

² Seegal, B. C., Seegal, D., and Khorazo, D., *J. Immun.*, 1933, **25**, 207.

* When the animal has been exsanguinated there can be only a trace of blood in this juice. The relative absence of blood is indicated by the fact that the tissue extract is not colored with hemoglobin which is the case with the fluid extracted from an organ which contains gross blood.

³ Seegal, B. C., The accompanying paper on "A Method of Obtaining Undiluted Tissue Juice for the Study of Tissue Antibody Titer."

TABLE I.
Typhoid agglutinin titer of serum, anterior chamber fluids and extracted tissues of the eye following the injection of a vaccine of *Eb. typhi* into the anterior chamber of the right eye; also similar tests in a series of control animals.

Animal No.	Preliminary treatment	Place of injection	Days	Control sample		Serum	Rt. ant. ch. fl.	Lt. ant. ch. fl.	Tissue juices	
				serum	Rt. ant. ch. fl.				right eye	left eye
515	Typhoid vac.	Right ant. chamber	4	0	0	1-40	0	0	0	0
513	"	"	6	0	0	1-64	0	0	1-8	0
516	"	"	8	0	0	1-512	1-16	0	1-16	0
514	"	"	12	0	0	1 1024	1-512	1-8	1-256	1-64
227	"	"	35	--	0	1-320	1-80	0	1-160	1-40
228	"	"	37	--	0	1-1280	1-20	0	1-640	1-320
511	"	"	46	0	0	1-1024	1-128	0	1-2920	1-64
231	"	"	49	--	0	1-160	1-80	0	1-320	1-40
1310	"	"	50	1-16	0	1-512	1-512	0	1-2048	1-16
229	"	"	84	--	0	1-64	0	0	1-64	1-8
230	"	"	98	--	0	1-64	1-64	0	1-256	1-16
512	"	"	102	0	0	1-640	1-32	0	1-2048	1-64
1	None	--	--	--	--	--	--	--	0	0
2	"	--	--	--	--	0	0	0	0	0
3	"	--	--	--	--	0	0	0	0	0
1312	Horse serum	--	--	--	--	1-512	0	0	1-16	1-16
583	"	Subcut.	30	--	--	1-16	0	0	0	0
584	"	"	32	--	--	0	0	0	0	0
2521	Typhoid vac.	"	8	--	--	1-512	0	0	1-16	1-16
1615	"	Intraven.	10	0	0	1-4000	1-16	1-32	1-1000	1-1000
1627	"	"	19	0	0	1-8000	1-32	1-32	1-2000	1-2000
4	"	Subcut.	60	--	--	1-160	1-6	1-6	1-40	1-40
2603	"	Intraven.	240	--	--	1-800	1-8	1-8	1-256	1-256

Agglutinin Titer in

agglutinin titer was higher than that of any of the other fluids tested. The titer of the anterior chamber fluid of the injected eye was variable but never higher than that of the serum. Agglutinins failed to appear in the anterior chamber fluid of the uninjected eye.† The tissue juices extracted from the iris, conjunctiva and sclera of the injected eye gave a titer of agglutinins which surpassed that found in the tissue juices of the normal eye but for the first month this titer did not surpass that of the serum. In those animals sacrificed from the 46th to the 102nd day, however, the antibody titer of the tissue juices extracted from the injected eye was equal to or greater than that of the serum. It also surpassed the antibody titer of the noninjected control eye 8 to 128 times. These results indicate that there is a concentration of specific agglutinins in the fluids of the tissues surrounding the anterior chamber of an eye which has been injected with typhoid vaccine. The data do not answer the questions concerning the site of formation of these antibodies and the reason for their concentration around the place of injection of the vaccine. (See Cannon and Sullivan⁶ for discussion.)

The results obtained with the control animals 1615 and 1627 are interesting since they offer an explanation for the observation that the repeated *intravenous* injection of a foreign protein may result ultimately in a hypersensitivity of the eyes without any *local* sensitizing injection of the antigen.⁷ These two rabbits had received intravenous injections of typhoid vaccine over a period of a month and a half. The antibody content of the tissue fluids from both eyes was so high as to suggest that the intravenous injection of the antigen in these animals might produce local inflammation in these tissues.

The present experiments have demonstrated that the injection of typhoid vaccine into the anterior chamber of the rabbit's eye results in a concentration of agglutinins in certain tissues of the eye. Twelve days after the intraocular injection of the antigen the titer of agglutinins in the juices extracted from these eye tissues begins to surpass that of the juices extracted from the opposite control eye. One to 3½ months after injection the titer of agglutinins in the tissue juices of the injected eye is equal to or greater than the agglutinin

† These observations agree with the findings of Madison⁴ and fail to confirm those of Schamburow⁵.

⁴ Madison, R. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1933, **30**, 1013.

⁵ Schamburow, D. A., *Z. f. Hyg. u. Infektionskrank.*, 1932, **114**, 456.

⁶ Cannon, P. R., and Sullivan, F. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1932, **29**, 517.

⁷ Seegal, B. C., and Seegal, D., *J. Immun.*, 1933, **25**, 221.

titer of the serum and is on an average about 12 times as great as the agglutinin titer of the control eye tissue juice.

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An Internal Electrocardiographic Lead.*

ABRAHAM LIEBERSON AND FRANK LIBERSON. (Introduced by Nathan F. Blau.)

From the Cardiac Research Division, United States Public Health Service Hospitals, New York.

The increased interest in chest and precordial leads, which are semi-direct leads tapping the cardiac action current from points close to the anterior surface of the heart, prompted the authors to seek an equivalent lead portraying more intimately the electrical phenomena at the posterior surface and base of the heart. A lead taken from the back of the chest fails to do this, for unlike the front, where there is only $\frac{1}{2}$ inch of tissue intervening between heart and electrode, there is usually 4 inches of intervening tissue posteriorly.

The difficulty was overcome by making use of the fact that the esophagus is closely attached to the posterior pericardium throughout its extent. A non-polarizable German Silver electrode, shaped somewhat like the Rehfuss tip, to which a thin flexible German Silver cable was clamped, served as an internal electrode. The cable was insulated throughout its length by rubber tubing. This electrode was swallowed in turn by 6 normal subjects, very much like the Rehfuss tube, its descent being checked by fluoroscopic examination. The esophageal electrode was connected to the right-arm side of the galvanometer, and the left-arm electrode (a flat 1 inch disc) lead to some point distant from the heart, such as the left leg or right arm of the subject.

When the internal or exploring electrode lay at the level of the auricles, the electrocardiogram demonstrated uniformly a peculiar deflection, a very sharp, steep, rapidly moving deflection, which arose from the rounded portion of the P-wave, and which was almost as tall as the QRS deflection, which it resembled. (See Fig. 1.)

The high complex described above is interpreted as the "intrinsic"

*Approved for publication by the Surgeon General, U. S. Public Health Service.