91. Sym: About Heterochromia Iridum, Ophth. Rev., 1889, viii, p. 202.
92. Sym: Review of Scalinci's Article, Ophth. Rev., 1915, xxxiv, p. 167.
93. v. Szily: Über die Entstehung des melanotischen Pigments, usw., Bonn, 1911, Cohen.
94. Thorpe: Case of Congenital Iridodialysis with Heterochromie, Brit. Med. J., 1906, ii, p. 140.
95. Waardenburg: Heterochromia of Iris, Nederl. Tijdschr. v. Geneesk., 1920, i, p. 1929; Ab. J.A.M.A., 1920, lxxv, p. 644.
96. Walther: Siehe Hess, Zur Theorie der Starbildung, Graefe-Saemisch Handbuch, Ed. 2, vi, p. 86.
97. Weill: Über Heterophthalmus, Ztschr. f. Augenh., 1904, xi, p. 165.
98. Wessely: Augenärztliche Erfahrungen im Felde, Würzburger Abhandl. a. d. Gesamtgeb. d. prakt. Med., 1915, xv, No. 9, p. 167.
99. Wilbrand und Saenger: Neurologie des Auges, Ergänzungsband I. Teil, 1927, p. 253.
100. Wilde: Dublin Quarterly Journal, November, 1848.
101. Williams: Heterochromia Iridis, Ophth. Rec., 1913, xxii, p. 361.
102. Würdemann: Hereditary Reversion Pigmentation of Eyelids with Heterochromia of Iris, Am. J. Ophth., 1920, iii, p. 874.

A FUNCTION OF BLOOD IN CORNEAL VASCULARIZATION*

F. H. HAESSLER, M.D.
Milwaukee

In recent years much excellent work has been done to show the unity of tissue reaction. By means of very simple physical and chemical reagents the relation of stimulus and response has been worked out. For example, in certain fish embryos the rate of the heart beat has been shown to be directly proportional to the temperature of the water. In speculating as to the causes of vascular proliferation it seemed possible that some substance present in the inflammatory lesion or in the blood itself might prove to be the fundamental stimulant.

This study was therefore undertaken with the hope of determining the causes of vessel growth. It is a well-known fact that extravasated blood in the retina or vitreous may in time cause proliferation of the blood-vessels. In the cornea, likewise, inflammatory lesions are accompanied by exudate and newly formed vessels. As the cornea is, under

* Candidate's thesis for membership accepted by the Committee on Theses.

normal conditions, completely avascular, it seemed as though here blood-vessel development could be studied to best advantage.

The first question, then, to be answered was, can blood or any of the constituents of blood alone cause vascular new growth? With this in mind, the eyes of a series of rabbits were injected first with fibrin, then with plasma, and finally with whole blood. In no instance did corneal vessels develop in response to these stimulants used alone.

A killed culture of staphylococcus aureus was next tried. This is, of course, a complex substance, and if it proved successful in bringing about vascularization, further study was contemplated to disclose just what factor was responsible for the vessel growth. However, no vascularization resulted.

Next, a mixture of whole blood and staphylococcus suspension was tried. The same killed culture, which alone had proved unsuccessful, with the addition of blood, produced corneal vascularization with almost constant success. The problem thus proved to be more complex than at first had been believed. However, it led to the suggestion that a common factor present in hemorrhage and exudate, namely, a coagulable protein, might act as an agent responsible for holding the irritant in contact with the tissue long enough to allow for vascular new growth.

To test the latter hypothesis, the killed culture of staphylococcus was mixed with nutrient agar, such as is in common use in a laboratory. This mixture also produced vascularization almost constantly.

DETAILS OF EXPERIMENTS

Intracorneal injection is easily accomplished with a No. 26 platinum needle, ground down as shown in the figure, which represents a cross-section of the needle before and after grinding. After making the cornea insensitive by cocain dropped into the conjunctival sac, the operator steadies the rabbit's head, separates the lids with one hand, and manipu-

lates a syringe armed with the specially ground needle with the other. No attempt need be made to hold the eyeball with fixation forceps while the needle is thrust into the cornea. With very little experience one is able to judge the depth to which the needle has penetrated. Usually the rabbits hold sufficiently still so that one is able to inject slowly and deliberately; thus one can produce any size bleb desired or modify the depth of the injection if the bleb is seen to be too superficial.

Fig. 1.—Cross-section of needle, as manufactured and as ground down for intracorneal injection.

REAGENTS AND THEIR PREPARATION

The following reagents were used:

1. A solution of pure fibrin.
2. Whole uncoagulated blood plasma of rabbits.
3. Whole blood of rabbits.
4. A suspension of formalin-killed staphylococcus aureus.
5. Whole blood mixed with staphylococcus suspension.
6. Nutrient agar.
7. Nutrient agar mixed with staphylococcus.

Though, on the whole, the names of the materials injected describe them sufficiently, a few notes as to their preparation and the reaction of the cornea to them seem necessary.

Fibrin was prepared from the clot of beef blood, washed in tap water until all color had disappeared, dissolved in 0.2 per cent. sodium hydroxid, filtered through cotton, precipitated by neutralization with normal acetic acid solution, thoroughly washed in distilled water, and kept in a dry state. For injection a solution was prepared by saturat-

ing a 0.05 per cent. sodium hydroxid solution with dried fibrin flakes.

In the three rabbits used this solution disappeared from the cornea in less than twenty-four hours. Another injection similarly given again disappeared in less than twenty-four hours.

Plasma was obtained by bleeding a rabbit from the heart into an oiled syringe and quickly centrifuging in a chilled paraffined tube. The supernatant plasma must be injected as quickly as possible. It clotted in the cornea, presumably, but disappeared without residuum in two or three days. Whole blood was obtained with a syringe from heart or ear vein and injected intracorneally before clotting. This also disappeared in two or three days except for a transparent greenish deposit. In a few rabbits a clot of whole blood was kept constantly present in the cornea for a period of three weeks by injecting more whole blood into the same place at intervals of three to five days whenever it seemed likely that the fibrin was disappearing. A suspension of a twenty-four-hour culture of staphylococcus aureus on agar was killed by the addition of 0.5 per cent. formalin and cultured after washing with salt solution to test for sterility. The suspension looked like a fairly heavy autogenous vaccine, but the necessity for laborious standardization was avoided by using the same suspension in all experiments. Actually such suspensions were made twice, as the time elapsing between experiments with blood mixtures and agar mixtures was great, but separate controls were injected in each series to demonstrate that neither of the staphylococcus suspensions by itself stimulated vascular proliferation.

To obtain a mixture of blood and staphylococcus, 1 c.c. of this killed bacterial suspension was placed in a 5 c.c. Record syringe which was subsequently filled to the 5 c.c. mark with blood directly from a rabbit's heart. To be sure, the injection of blood mixtures was not always successful but only

those rabbits were considered as part of this series in which a satisfactory injection was made.

The agar was that used in the laboratory for cultures. Before use it was melted in boiling water and cooled to 40° C. The mixture of agar and staphylococcus consisted of four parts agar and one part bacterial suspension. When vascular stimulation resulted, the vessels arose from the superficial conjunctival network in the limbus. The vessels appeared within eight days after injection.

The appended table lists the experiments performed and the results.

SUMMARY

Solutions of fibrin, blood plasma, and whole blood were injected into rabbits' eyes without stimulating corneal vascularization. Injections of whole blood repeated sufficiently often to keep a visible amount constantly present for three weeks were equally unsuccessful. The addition of a killed culture of staphylococcus aureus caused rapid vascularization of the cornea, while the cornea of the other eye in the same animal injected with the same staphylococci suspended in salt solution cleared rapidly and remained free from vessels. This result suggested that the blood acted mechanically in holding the irritant in the corneal tissue for a sufficient length of time to remain a stimulus for the growth of vessels from the limbal network. To test this hypothesis, the eyes of a series of rabbits were injected with a suspension of killed staphylococcus. Control injections of staphylococcus and of agar, each alone, were also done. It was found that neither substance by itself stimulated vascularization, but that the mixture did so in five of six eyes.

CONCLUSIONS

One of the functions of blood, or more probably its clot, is to increase the time during which the substance

Rabbit No.	Right eye injected with	Left eye injected with	Vascular Proliferation Produced	
			O. D.	O. S.
1	Fibrin sol.	..	0	
2	" "	..	0	
3	" "	..	0	
4	Blood plasma	..	0	
5	" "	..	0	
6	" "	..	0	
7	" "	..	0	
8	" "	..	0	
9	" "	..	0	
10	Whole blood	Whole blood	0	0
11	" "	" "	0	0
12	" "	" "	0	0
13	" "	" "	0	0
14	" "	" "	0	0
15	" "	" "	0	0
16	" "	" "	0	0
17	" "	" "	0	0
18	Whole blood repeatedly for 3 weeks	..	0	
19	Whole blood repeatedly for 3 weeks	..	0	
20	Whole blood repeatedly for 3 weeks	..	0	
21	Whole blood and staphylococcus	Suspension of formalin-killed staphylococcus	+	0
22	Whole blood and staphylococcus	Suspension of formalin-killed staphylococcus	0	0
23	Whole blood and staphylococcus	Suspension of formalin-killed staphylococcus	+	0
24	Whole blood and staphylococcus	Suspension of formalin-killed staphylococcus	+	0
25	Whole blood and staphylococcus	Suspension of formalin-killed staphylococcus	+	0
26	Agar and staphylococcus	Agar	+	0
27	" " "	"	+	0
28	" " "	"	+	0
29	" " "	"	0	0
30	" " "	"	+	0
31	" " "	"	+	0
32	Staphylococcus	Staphylococcus	0	0

which stimulates vascular proliferation remains in the cornea.

The constituents of blood, acting alone, do not stimulate vascular proliferation into the cornea.