

CHANGES IN BLOOD VESSELS (CAPILLARY FRAGILITY) WITH INFLAMMATION

By ERNST ZANDER

(From the Department of Pathology, Cornell University Medical College and New York Hospital, New York)

(Received for publication, August 5, 1937)

The occurrence of hemorrhage in association with many forms of inflammation has suggested a study of changes in the vascular walls that favor the escape of red blood corpuscles. Inflammation of the cutis has been produced by a variety of means and the ability of the vessels to withstand mechanical injury produced by suction applied to the skin surface has been determined.

Capillary bleeding can be produced by altering the relation between extra- and intracapillary pressure. When venous flow is obstructed by application of an elastic bandage to the arm intracapillary pressure is increased and petechiae occur. Rumpel (1) and Leede (2) discovered this phenomenon in patients with scarlet fever. Goethlin (3), Bayer (4), Bexelius (5), Walterhoefer (6) and others have developed methods of measuring capillary fragility based on this phenomenon. Goethlin, Shultzer (7) and others found a marked increase in capillary fragility in patients suffering from scurvy and a somewhat increased tendency to capillary bleeding in patients who had a history of vitamin C deficiency in their diet, as in patients fed on a diet of milk, cream and cereals used in the treatment of peptic ulcer. Walterhoefer found that the fall upon the forearm of a weight of 130 gm. from a height of 35 cm. increased the number of petechiae which occur when an elastic bandage is applied to the arm. Bayer obtained an increased number of petechiae when he applied linen saturated with hot water for 5 minutes before application of the elastic bandage. He described increased capillary fragility in skin areas of slight erythema produced by ultraviolet light. Bexelius found the number of petechiae somewhat increased when the patient took a hot steam bath shortly before he applied the elastic bandage.

Hecht (8), Silva Mello (9), Dalldorf (10) and Cutter (11) produced capillary bleeding by application of a partial vacuum to the skin. They used this suction test to recognize changes in the blood vessels with vitamin C deficiency. They determined the lowest partial vacuum which suddenly applied to the skin produced petechiae, and counted their number.

In the following experiments, which have been done under the direction of Dr. E. L. Opie, suction is used as a measure of the injury required to produce hemorrhage. A vacuum applied to the skin of rabbits is slowly increased till the pressure reaches -70 mm. Hg and this level is maintained until a uniform dark red hemorrhage composed of innumerable petechiae has occurred over the whole area subjected to the suction. The time necessary to produce this effect is an approximate index of susceptibility of the capillary wall to hemorrhage, designated for convenience capillary fragility.

Fig. 1 shows the suction apparatus that has been used. It consists of a water pump, two 2000 cc. glass bottles (I and II), a 500 cc. separator (III), a mercury manometer and a glass tube to apply the suction to the skin, all connected by means of heavy walled rubber tubes and connecting glass tubes. A high partial vacuum is made in the reservoirs I, II and III by means of a water pump. A number of stop-cocks transfer this pressure slowly or suddenly to the suction tube that is applied to the skin at the desired point. The pressure in the reservoirs can be measured by the manometer by turning the 3 way cock D and opening stop-cock G. Stop-cock E is permanently adjusted so that air from the suction tube passes very slowly through it into vacuum reservoir III. By opening stop-cock F for about 1 minute the vacuum in the suction tube gradually increases until a pressure of -70 mm. Hg is reached. Continuity of the suction tube with vacuum reservoir I after stop-cock B is opened permits immediate production of vacuum in the suction tube. By opening stop-cock C air can be let into the suction tube and atmospheric pressure regained. The suction tube is a simple straight glass tube, 8 cm. long, 8 mm. in diameter and 1 mm. thick, carefully rounded at the end. Vaseline applied to the skin of the rabbit makes the junction between skin and tube air-tight. With the apparatus that has been described a vacuum applied to the skin remains at constant pressure for at least 1 hour.

The hair of the rabbit has been clipped 24 hours before testing because shaving produces strong irritation of the skin. Rabbits weighing more than 2000 gm. have been used. Suction with gradually increasing vacuum produces gradually increasing hyperemia of the normal skin. When the suction test is applied to smaller rabbits with thin skin the hyperemia occasionally fails to occur and the observations are unsatisfactory. It is found empirically by applying suction to the skin in several places on the same animal that a vacuum of -70 mm. Hg gives more constant results than suction with higher or lower pressures.

With normal skin of the rabbit petechiae usually appear after 4 to 5 minutes of suction and in most rabbits hemorrhage occurs after 8 minutes over the whole area subjected to the suction. There are only slight differences in the results of the test applied to skin in different

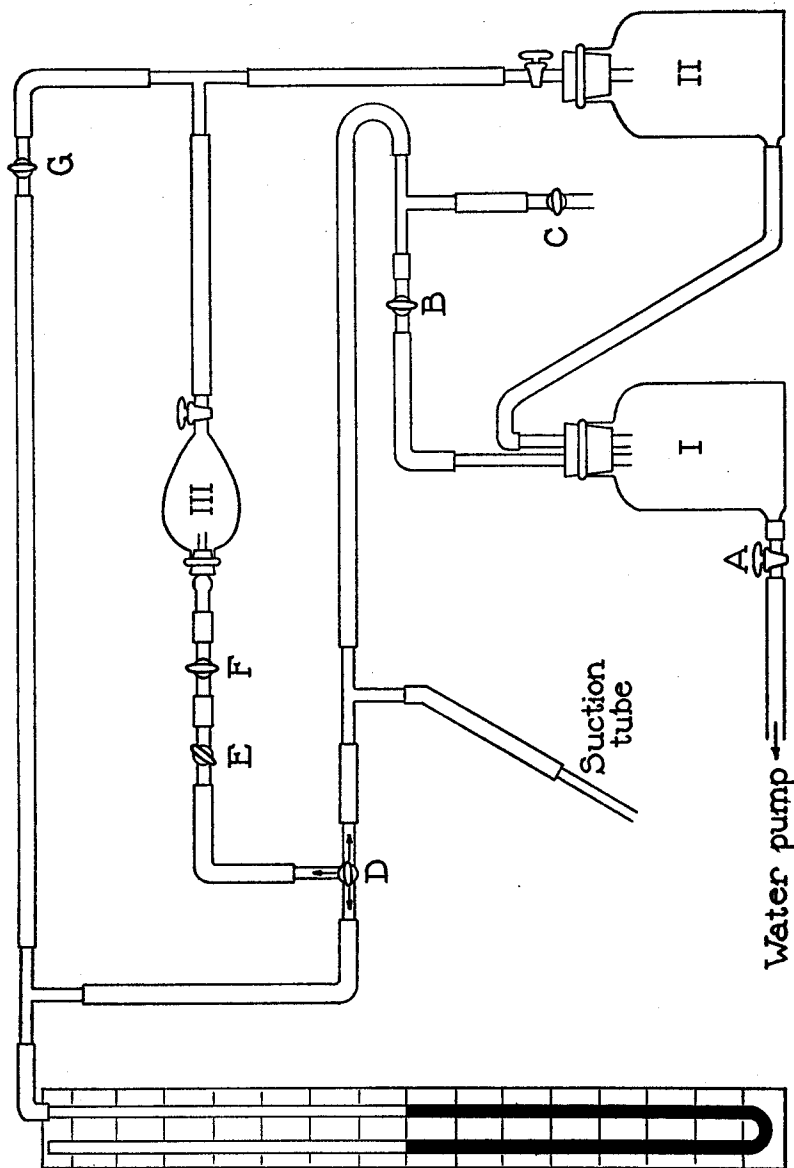


FIG. 1

places from the spine to the mammillary line. The suction provokes capillary bleeding more quickly when it is applied adjacent to previously made hemorrhages and tests should be made at least 3 cm. apart. Suction time in subsequent experiments is the time required to produce the vascular injury that is revealed by hemorrhage.

Suction causes hemorrhages into the skin of the legs after an interval of approximately 2 or 3 minutes longer than that required for their production on the flank of the animal. Hemorrhages in the skin of the ears are produced with difficulty by this method. In tests made on one animal daily for 7 days the time required for suction to produce hemorrhage varied from 9 to 12 minutes. 50 tests on 20 normal rabbits, designated 1 to 20, produced hemorrhage after the time intervals shown in the tabulation.

5 min.	6 min.	7 min.	8 min.	9 min.	10 min.	11 min.	12 min.	15 min.
Rabbit No.	Rabbit No.	Rabbit No.	Rabbit No.	Rabbit No.	Rabbit No.	Rabbit No.	Rabbit No.	Rabbit No.
11	1	3	2	2	7	8	6	14
	11	3	2	6	9	19	8	
	17	5	3	10	9		8	
	17	14	4	13	19		10	
	17	15	4		19		14	
	20	15	5					
	20	16	5					
	20	18	7					
		18	7					
			12					
			12					
			13					
			14					
			16					
			16					

Histological studies of the lesions produced in normal skin by suction of 8 minutes duration did not determine whether the capillary bleeding in the cutis was due to rupture or to diapedesis of red corpuscles.

Inflammation Produced by a Chemical Irritant

Changes in capillary fragility were studied at the site of inflammation produced by a chemical irritant. Turpentine was diluted with

paraffin oil 1:50, and 0.1 cc. of the mixture was injected intracutaneously at 10 sites on the flank of a rabbit. Redness and edema marked the site of inflammation during a period of several days. Suction applied to the normal skin produced hemorrhage after 12 minutes, but 30 minutes after injection suction had to be applied during 100 minutes to produce hemorrhage. Increased capillary fragility made its appearance 6 hours after injection when the suction time was 2 minutes and 30 seconds, and was still present after 24 hours with a suction time of 3 minutes. The capillary resistance against suction was approximately normal 48 hours after injection.

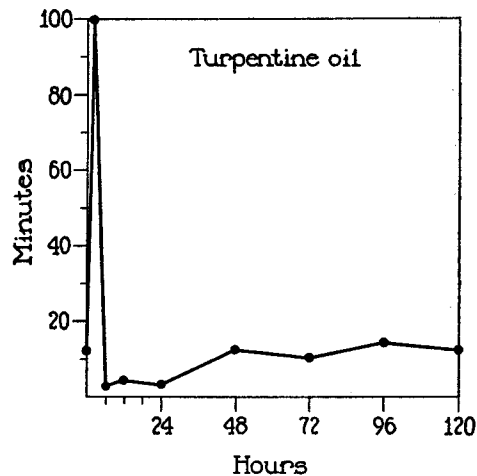


FIG. 2

Inflammation Produced by Bacteria

Inflammation was produced in the first of these experiments by injection of killed hemolytic streptococci into the skin. To a broth culture 24 hours old 0.2 per cent formalin was added and after centrifuging the sediment was suspended in saline up to the original volume of the culture. Intracutaneous injections of 0.1 cc. of the suspension were made at 10 sites. Suction tests were made by applying the suction tube to the central parts of injected areas at different time intervals after injection (Fig. 3, 1st injection). The skin became pale immediately after injection and remained white from 1 to 3 hours. 30 minutes following injection suction produced hyperemia after it

had been applied for 95 minutes and 25 minutes later hemorrhage appeared over the whole area of suction, but it was somewhat less

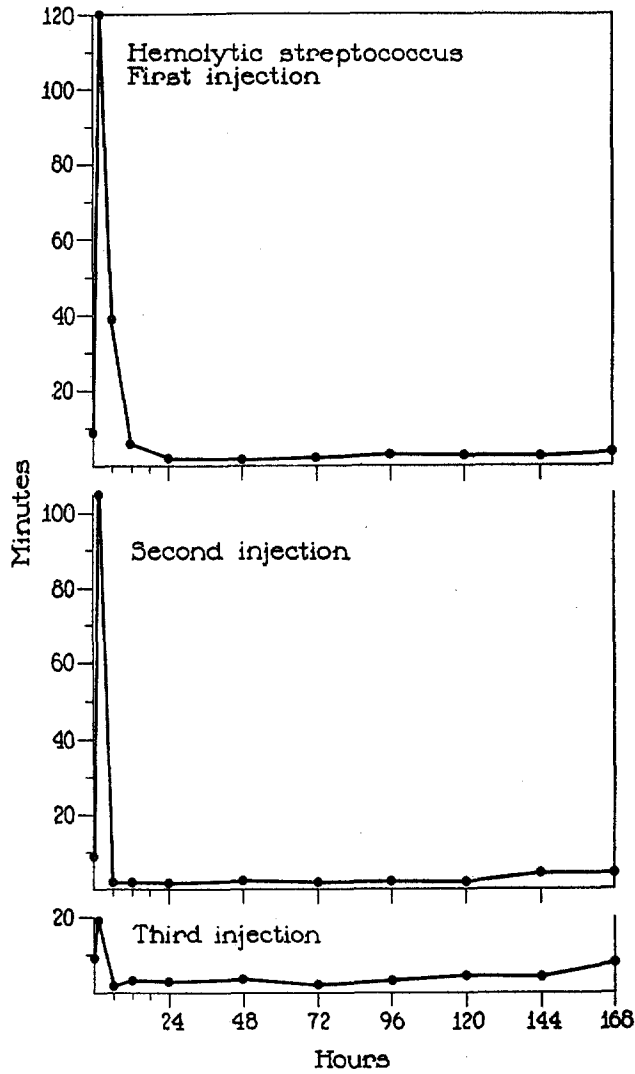


FIG. 3

uniform and usually brighter red than that which occurs in normal skin. There was no difference in the result of suction when a high

vacuum (-300 mm. Hg) or the usual low vacuum (-70 mm. Hg) was used. After 6 hours when redness and edema of inflammation had appeared resistance to suction slightly greater than normal still remained. 12 hours after the injection the suction time was reduced to 6 minutes and after 24 hours to 1 minute and 45 seconds. The increased susceptibility to bleeding with suction persisted for several days. The experiment was repeated on 5 rabbits and showed the same increase in capillary fragility which gradually disappeared after 6 to 9 days.

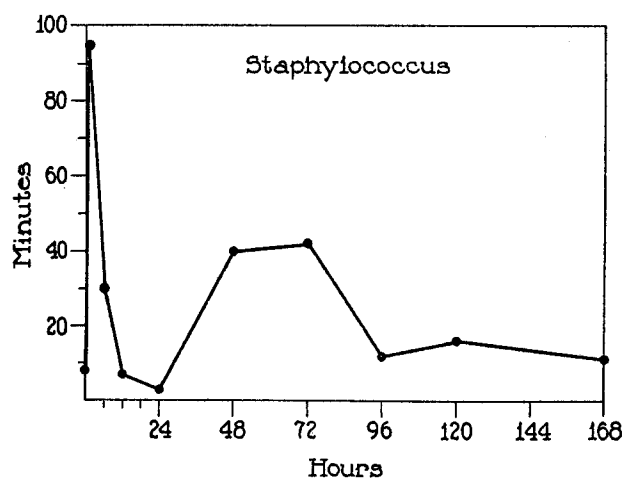


FIG. 4

Inflammation produced by injection of other heat-killed bacteria caused similar changes. A bouillon culture of *Staphylococcus aureus* 24 hours old was killed by heating to 60°C . for 30 minutes and 0.5 cc. was injected intracutaneously at each of 10 sites on the flanks of a rabbit. There was ill defined redness and edema after 24 hours. The suction test applied to the central parts of these lesions demonstrated (Fig. 4) that the time required to produce hemorrhage was greatly increased a half hour after the injection and was diminished below normal after 24 hours. Increased susceptibility to capillary bleeding disappeared after 48 hours. The time required to produce hemorrhage exceeded normal after 72 hours but later returned to approximately the normal level.

Inflammation produced by a suspension of heat-killed meningococci in saline solution containing approximately 2,000,000,000 bacteria per cc. was accompanied by similar changes in the rapidity with which hemorrhage was caused by suction. Intracutaneous injections of 0.2 cc. of the suspension produced redness and edema in 10 areas and these were tested by suction after different intervals following injection. There was prolongation of suction time (75 minutes) preceding hemorrhage 30 minutes after injection and an increase in capillary fragility after 12 and 24 hours with suction times of 2 minutes and 30 seconds and 4 minutes, respectively. The suction time had returned to normal (10 minutes) after 48 hours and after 72 hours

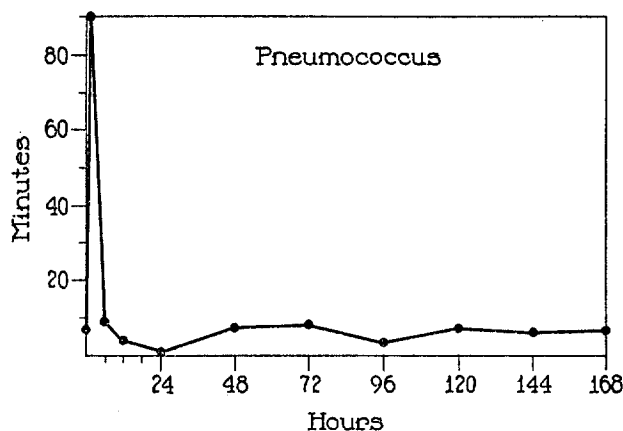


FIG. 5

exceeded this level (18 minutes) but later became approximately normal.

Each of two rabbits received ten intracutaneous injections of 0.2 cc. of a heat-killed bouillon culture of pneumococcus which was originally obtained from a human case and then passed several times through rabbits until it was highly virulent for these animals. The resulting areas of redness and edema after 48 hours measured about 12 mm. across and were elevated 2 mm. The results of the suction tests applied in the center of the lesions at the usual time intervals gave a curve (Fig. 5) similar to those of the foregoing experiments. Capillary resistance returned to normal after 48 hours.

In all these experiments greatly increased resistance of the capillaries to suction was observed immediately following the injection of a chemical or of heat-killed bacteria into the skin. It is probably due to contraction of the small cutaneous vessels stimulated by the injected irritant. It does not occur when a second injection of an inflammatory irritant is made into the site of acute inflammation, presumably because the vascular walls are paralyzed by the first injection. A heat-killed culture of *Staphylococcus aureus* was injected intracutaneously in a number of places each receiving 0.2 cc., and a test made after 30 minutes demonstrated the usual increase in capillary resistance to the production of hemorrhage by suction. After 24 hours 0.2 cc. of the same heat-killed culture was now injected into an area of inflammation produced by one of the preceding injections. After an interval of 30 minutes the suction test applied to the lesion showed no increased resistance to the production of hemorrhage by suction. The experiment was repeated twice with killed streptococci and the same result was obtained.

Allergic Inflammation

Allergic inflammation produced by repeated injection of an antigen is characterized by rapid appearance and increased intensity of various manifestations of inflammation (Opie, 12). Experiments have been undertaken to determine what changes in capillary fragility occur during the course of allergic inflammation produced by killed hemolytic streptococci.

The six rabbits that were used in the experiment already described (Fig. 3, 1st injection) received 10 days after the first injection 10 intracutaneous injections of 0.1 cc. of a similar streptococcus suspension. The suction test was applied to these lesions after time intervals that were the same as those in the first series (Fig. 3, 2nd injection). Sensitization was indicated by the increased size of the lesions, which after 48 hours were well defined, red and edematous and measured 11 x 10 x 2 mm. 30 minutes after injection there was prolongation of the suction time for hemorrhage only slightly less than that after the first injection. The conspicuous increase in susceptibility to capillary bleeding that appeared in inflamed areas of the first series of injections after 12 to 24 hours was found after 6 hours. 10 days later a third

series of 10 intracutaneous injections of the same amount of killed streptococcus was made in each of these rabbits. A half hour after injection, the primary prolongation of suction time required to produce bleeding was much less (Fig. 3, 3rd injection) than that observed in the lesions of the second series of injections, but it is not evident that this inhibition of resistance that ordinarily occurs soon after the injection of an inflammatory irritant is characteristic of sensitization. A more constant change was the rapidity with which increased capillary fragility made its appearance.

Similar changes in capillary fragility were found in another series of experiments in which rabbits were well sensitized to hemolytic streptococci. 9 rabbits received an intracutaneous injection of 0.1 cc. of a suspension of killed hemolytic streptococci daily for a week and no

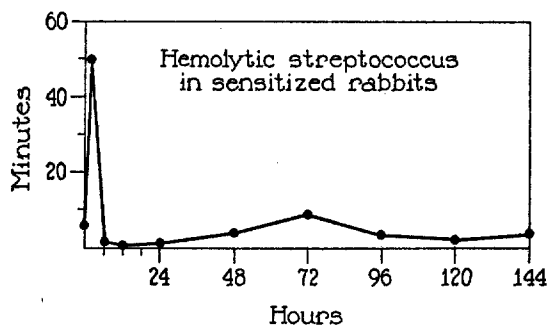


FIG. 6

injections during the following week. A 2nd week of daily injections was followed by a week of rest. During a 3rd week of daily injections suction tests were applied to the lesions that were produced by the first of these daily injections. The lesion of 1 rabbit was tested 30 minutes after the injection, the lesion of another rabbit after 6 hours, of a 3rd after 12 hours, of a 4th after 24 hours, of a 5th rabbit after 2 days, of a 6th after 3 days, of a 7th after 4 days, of an 8th after 5 days and of a 9th after 6 days. The same procedure was applied to the lesions produced by injections on the 2nd, 3rd, 4th, 5th and 6th day of this third series of daily injections. The average extent of redness and edema 48 hours after injection of killed streptococci was 25 mm. in diameter and elevated 5 mm.; necrosis occurred in the central part of some lesions. The test was applied at the edge of the lesions where there was no necrosis.

The graphs representing tests made upon lesions of each of the 6 days were similar. The changes in capillary fragility that occurred in lesions following injection on the 2nd day are shown in Fig. 6. The early appearance of advanced capillary fragility evident after 6 hours was a constant feature of the lesions in these sensitized animals. It was greatest at 12 hours, the whole area subjected to the suction becoming hemorrhagic after 40 seconds. The capillary resistance in these animals returned to normal a few days earlier than in animals that had not been sensitized by preceding injections of streptococci.

Injection of tuberculin into animals sensitized by injections of killed tubercle bacilli produced severe and long continued capillary damage.

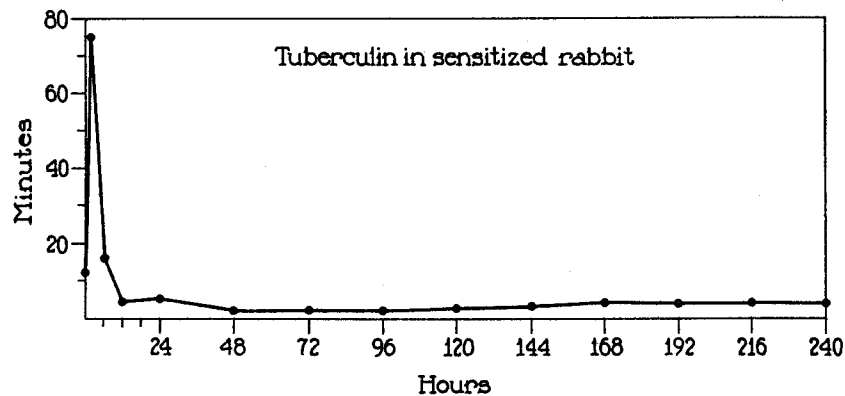


FIG. 7

2 sensitized rabbits received in the flanks 14 intracutaneous injections of 0.2 cc. of Old Tuberculin diluted 1:5 with physiological saline solution. In one rabbit after 48 hours there was ill defined redness and edema 32 x 22 mm., elevated 1 mm.; in the other rabbit 15 x 12 mm., elevated 1 mm. The results of the suction tests were essentially the same in the two animals. After the usual primary retardation of bleeding, increased capillary fragility made its appearance but was not greater than that with inflammation in unsensitized animals. This increase of capillary fragility persisted during 2 weeks. The last two tests are not shown in Fig. 7.

It is noteworthy that the resistance of capillaries to suction with allergic inflammation undergoes changes that are essentially the same

as those with inflammation in normal animals. In animals sensitized to streptococci increased fragility has made its appearance sooner than in normal animals, but with the reaction to tuberculin in sensitized animals this acceleration has not been found.

Shwartzman Phenomenon

The Shwartzman phenomenon (13-15) occurs when an injection of certain toxic bacterial filtrates into the cutis is followed after 24 hours by the injection of the same substance into the circulating blood. A hemorrhagic lesion occurs at the site of the preparatory injection. Experiments were undertaken to determine if changes in capillary fragility at the site of this skin-preparatory injection render the tissues here susceptible to hemorrhage.

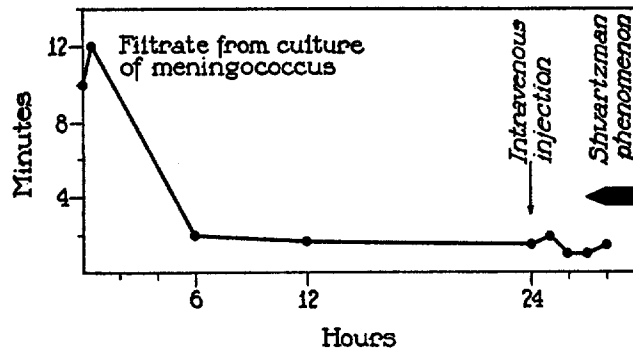


FIG. 8

Meningococcus filtrate was prepared by Dr. Jules Freund according to the method recommended by Shwartzman. 0.1 cc. of diluted filtrate (1 part filtrate and 2 parts physiological saline solution) was injected intracutaneously at 9 sites. Suction tests were applied at the edges of these lesions after it was found that the edema in the center protected the vessels from suction. Prolongation of the suction time 30 minutes after injection was unusually slight. Greatly increased capillary fragility was evident at 6, 12 and 24 hours, the suction times required to produce hemorrhage being respectively 2 minutes, 1 minute and 50 seconds and 1 minute and 20 seconds. At the sites of the preparatory injection after 24 hours edema and redness were approximately 15 mm. across and elevated 1 mm. At this time 1 cc.

of the undiluted filtrate was injected intravenously. 1 hour following the intravenous injection 2 of the 9 injected areas showed a slight purple discoloration and after about 3 hours all of them were the sites of hemorrhage of varying intensity.

In a second experiment in which intracutaneous injections of 0.1 cc. of diluted (1:6) and an intravenous injection of 1 cc. undiluted meningococcus filtrate were used to produce the Shwartzman phenomenon similar increase in capillary fragility was associated with the occurrence of hemorrhagic lesions. In this experiment the preparatory lesions were less edematous and suction applied in the center of them produced hemorrhage $\frac{1}{2}$ hour after injection in 10 minutes, after 6 hours

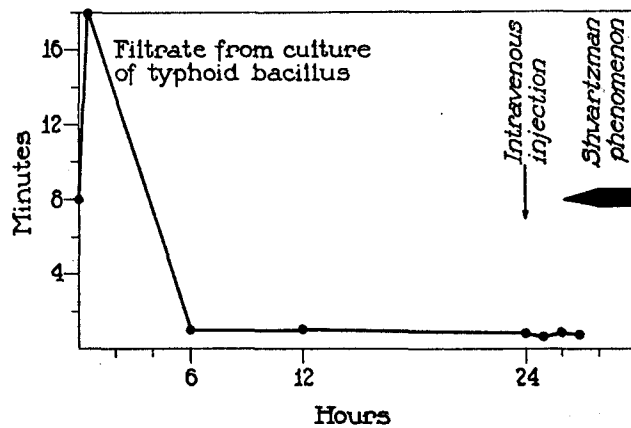


FIG. 9

in 2 minutes and 30 seconds and after 24 hours in 1 minute and 40 seconds.

A similar experiment was made with typhoid filtrate prepared according to the method recommended by Shwartzman. 2 rabbits received intracutaneous injections of 0.2 cc. of the filtrate at 8 sites and the suction test was applied at the usual intervals. The preliminary prolongation of suction time was less than that usually observed. Greatly increased capillary fragility was found after 6 hours, suction time being 1 minute and 30 seconds, although little edema was evident at this time. Unusual susceptibility to capillary hemorrhage could be demonstrated 12 and 24 hours after injection, the suction time after

24 hours being 40 seconds in the edematous lesions now 20 mm. in diameter and elevated 1 mm. After 2 hours following the intravenous injection of 4 cc. of the same filtrate, purple discoloration began to appear in these lesions and hemorrhage became evident.

The preparatory injection used to produce the Shwartzman reaction produces changes in capillaries that increase their susceptibility to hemorrhage. These changes follow a sequence that is the same as that observed with other forms of inflammation (Koplik, 15), but capillary fragility produced by substances that elicit the Shwartzman phenomenon is unusually great. It is probable that the intravenous injection reaches by way of the blood stream injured capillaries at the site of inflammation produced by the preparatory injection and intensifies this injury so that hemorrhage results.

CONCLUSIONS

Suction with a partial vacuum of -70 mm. Hg applied to normal skin of rabbits causes intracutaneous hemorrhage after an average time of 8 minutes.

Inflammation produced by various agents, including turpentine, killed streptococci, staphylococci, pneumococci or meningococci, and filtrates from cultures of meningococci or typhoid bacilli, produces a series of changes that are almost uniform.

Immediately after injection of the irritant there is greatly increased resistance to the production of hemorrhage by suction so that the time required may be from $\frac{1}{2}$ hour to almost 2 hours. This increased resistance to suction applied to the surface of the skin is doubtless caused by contraction of blood vessels following injection of the irritant.

The period of increased resistance is soon followed by diminished resistance of the vascular walls so that hemorrhage after 12 to 24 hours following injection occurs within from 1 to 4 minutes of suction.

The subsequent course of events varies; resistance of the vascular wall to suction, designated for convenience capillary fragility, may return to normal after from 2 to 9 days (observed with turpentine, streptococcus), or for several days may considerably exceed this level (observed with staphylococcus, pneumococcus), or may remain at a low level for a week or more (observed with tuberculin).

With inflammation in a sensitized animal (allergic inflammation) the preliminary period of resistance may be diminished and the appearance of capillary fragility hastened, so that hemorrhage occurs after 2 minutes of suction applied 6 hours after injection, and later it may fall to an even lower level (observed with hemolytic streptococci in sensitized animals).

The preparatory injection of toxic substances, such as meningococcus or typhoid filtrate, used in the production of the Shwartzman phenomenon, causes inflammation with injury of small blood vessels indicated by susceptibility to hemorrhage with suction. It is probable that subsequent intravenous injection causes hemorrhage by further injury to these injured blood vessels.

BIBLIOGRAPHY

1. Rumpel, *Münch. med. Woch.*, 1909, **56**, 1404.
2. Leede, C., *Münch. med. Woch.*, 1911, **58**, 293.
3. Goethlin, E., *Skand. Arch. Physiol.*, 1931, **61**, 225.
4. Bayer, W., *Jahrb. Kinderheilk.*, 1930, **128**, 311; 1930, **129**, 55.
5. Bexelius, G., *Acta med. Scand.*, 1933, **80**, 281.
6. Walterhoefer, *Münch. med. Woch.*, 1925, **43**, 1819.
7. Shultzer, P., *Acta med. Scand.*, 1934, **81**, 113.
8. Hecht, A. F., *Jahrb. Kinderheilk.*, 1907, **65**, 113.
9. Silva Mello, A., *Münch. med. Woch.*, 1929, **76**, 1717.
10. Dalldorf, G., *Am. J. Dis. Child.*, 1933, **46**, 794.
11. Cutter, S., *J. Am. Med. Assn.*, 1935, **105**, 505.
12. Opie, E. L., *Medicine*, 1936, **15**, 4.
13. Shwartzman, G., *Proc. Soc. Exp. Biol. and Med.*, 1928, **25**, 560; 1931, **29**, 193; *J. Exp. Med.*, 1933, **57**, 859.
14. Freund, J., *J. Exp. Med.*, 1934, **60**, 661.
15. Koplik, L. H., *J. Exp. Med.*, 1937, **65**, 287.