PERIPHERAL VASCULAR REACTIONS IN ANAPHYLAXIS OF THE MOUSE

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In the course of studies on the site of antibody formation it was found that mice sensitized to horse serum showed extraordinary vascular reactions in the ears when reinjected with the same serum at an appropriate interval. The phenomena, observed under the microscope, appeared not only in animals showing anaphylactic shock but also in many which presented no other apparent signs of anaphylaxis. That is to say the vascular changes in the ear seemed to constitute a sign of anaphylactic sensitivity far more delicate than the production of anaphylactic shock itself. It seemed likely that these reactions could be used for the study of the mechanisms of local hypersensitivity. Further, it seemed probable that by means of these reactions the mouse could be used as a convenient laboratory test animal to take the place of larger or more costly animals for various immunological studies.

Because of erroneous statements in the older literature (1-7) it is still believed, even by many immunologists and allergists, that the mouse is not susceptible to anaphylactic shock, and the possibility of using the vascular changes in its ears for immunological research has, of course, not been considered. Under these circumstances it seemed wise to make a study of the characteristics of the peripheral vascular responses in mice showing anaphylactic shock in all degrees of intensity, not only to learn more about them but to determine whether they are a true part of the anaphylactic reaction—and hence an indication of the animal's sensitivity—or whether they are merely secondary to blood pressure changes or nerve stimuli.

The present paper describes the findings of such a study carried out in mice sensitized to various sera and reinjected later with the same material. The vascular changes will be fully described since they have brought out some new findings of physiological interest quite apart from their immunological implications.

Although a number of workers (8–17) have observed anaphylactic shock in the mouse their studies have not been aimed at an understanding of the circulatory changes taking place in this animal. On the contrary little is known about this matter. Fortunately for us methods for observing the most minute as well as the larger blood vessels in the ears (18) or claws (19) of mice, and for measuring their blood pressure during and after anaphylactic shock were already at hand in this laboratory (19).

Previous Work.—In spite of the fact that several authors (1–7) reported between 1908 and 1910 that mice are refractory to anaphylactic shock, papers by Braun (8, 9) and Schultz and Jordan (10) appeared within about a year of each other, proving the contrary. Shortly thereafter Ritz (11) and von Sarnowski (12) also produced anaphylactic shock in mice, and in 1926 Schiemann and Meyer (13) obtained both active and passive anaphylaxis. In 1937 Bourdon (14) reported active sensitization of white mice and recently Weiser, Golub, and Hamre (15) have restudied the subject and discussed the previous findings. The mouse has also been used by Mayer and Brousseau (16) and also by Perry and Darsie (17) to gage the activity of antihistaminic drugs and to study histamine shock.

The Induction of Anaphylactic Shock in Mice

In the present experiments trial and error showed that mice could be rendered anaphylactically sensitive, as a rule, by injecting 0.03 cc. of horse, pig, or rabbit serum into the peritoneal cavity twice, at an interval of 48 hours. After an appropriate period shock was induced by injecting into a tail vein 0.05 to 0.15 cc. (usually 0.1 cc.) of the sensitizing serum, per 30 gm. of body weight. Optimum results were obtained when the mice were shocked 16 to 35 days after the first sensitizing injection. Under these circumstances about three-fourths of the mice showed the pronounced shock symptoms that will be detailed below. About 20 per cent of them died 20 minutes to 2½ hours after the injection, and another 10 to 20 per cent died during the next 12 hours. The reactions were less regular and less severe when shorter or longer time intervals elapsed between the sensitizing and shocking injections, but shock was obtained as early as the 9th day and as late as 6 months after the first sensitization. Longer intervals have not been tried. Usually, however, shocking injections made at an interval of more than 7 weeks gave irregular findings.

Types of Experiments

Normal white mice of the Rockefeller Institute strain were employed throughout. Both unanesthetized and anesthetized animals were shocked in order to correlate the time relationship between the physiological changes, which could be best observed in the latter, and the behavior phenomena displayed by the former. Single intraperitoneal injections of pentobarbital, 0.5 cc. of a 1 per cent solution per 30 gm. of body weight, yielded suitable anesthesia with which to observe the circulatory changes in the ears or claws and to measure the blood pressure changes in the carotid and femoral arteries. These observations were made before, during, and after shock, by methods to be outlined below. The blood pressure measurements could, of course, be carried out only in the anesthetized animals, but it should be stressed here that the vascular changes, observed to the best advantage in the anesthetized mice as described further on, were also observed, with some difficulty, taking place in unanesthetized ones, at the same times after the injection of antigen and with the same intensity as in the anesthetized animals. It follows that anesthesia was not responsible for the findings.

Signs of Anaphylactic Shock in Unanesthetized Mice

The earlier workers are agreed (8–17) that anaphylactic shock in the mouse is a less stormy event than in the guinea pig or rabbit. For the first 5 or 10 minutes unanesthetized mice show merely agitation or hyperexcitability (15). Later they scratch themselves (10), the hair becomes ruffled, respiration is difficult, and some throw themselves here and there (12) or have brief convulsions with long, quiet periods between (15). Cyanosis in the ears and feet has been noticed (12, 15), but a prior blanching has not been mentioned although, as reported below, it appears regularly soon after the shocking injection is given. There is often a frog-like posture with the hind legs extended behind the body (15). Death does not come on

rapidly as in shocked guinea pigs and rabbits, but after 15 to 60 minutes (15), or it may occur after many hours.

We have found the picture of shock induced by horse, pig, or rabbit serum in unanesthetized mice substantially like that described above, with one great exception. Many of the animals showed either no signs when observed in the gross, or at most only agitation and restlessness; but when their ears were examined at low magnification after they had been placed in a holder, one observed striking vascular reactions like those now to be described as occurring in perfectly quiet anesthetized mice. They differed only in degree, being less severe, than those seen in mice with outspoken anaphylactic shock.

PERIPHERAL CIRCULATORY CHANGES TAKING PLACE IN ANESTHETIZED MICE WITH ANAPHYLAXIS

In contrast to the delay in the appearance of the manifestations of anaphylaxis, the microscope revealed profound physiological changes in the blood vessels of the ears and claws taking place promptly. They appeared even during the injection of antigen, and long before unanesthetized animals showed any signs of distress.

Changes in the Circulation of the Ears.—In more than 300 experiments anesthetized normal or sensitized mice, approximating 30 gm. in weight, were placed prone in plastaline moulds with their ears spread out upon white porcelain placques, in a manner previously described (18–20). By these means even the most minute, as well as the larger, vessels were observed in the intact ears under the microscope. Next, one worker injected 0.05 to 0.15 cc. of serum into a tail vein while another observed the ear vessels during the injection and for various periods thereafter. The injection required 30 seconds to 1 minute.

At any time from 28 seconds to 7 minutes after beginning the injection, but usually between the 50th and 90th seconds, vascular reactions began, if the animals were sensitive.

Changes in the Arteries.—Usually there appeared first one or more brief, partial contractions extending all along the arteries of the mid and peripheral portions of the ears. In a second or two the vessels returned to their initial calibers. Momentarily the circulation increased in speed, but then, after a few seconds, it became much slower than it had been before the injection. Next, sharply localized contractions appeared in many arteries. Some promptly relaxed, but others persisted. In highly sensitive animals the localized spasms increased in number and intensity, until, within a minute or two, all arterial vessels were completely constricted and had disappeared from view. In less sensitive mice only some of the arteries showed a complete obliterative spasm while in the others the local constrictions remained, trapping blood in the vascular segments lying between them. In poorly sensitized mice only a few localized spasms were seen. More will be said of these differences below.

In those instances in which vascular spasm did not occur for a minute or two, the slowing of the circulation was the most prominent feature. Cells, moving in clumps, separated by plasma could be seen, as though they had become sticky and adherent. In rare instances flow ceased in ear arteries which were still patent, showing that spasm must have occurred in larger, more centrally situated arteries than those of the ear. In the vessels that had become completely occluded blood flow stopped of course, but in the vessels in which constriction was partial the flow merely remained slow.

Changes in the Veins.—The veins also contracted. Usually sharply localized, ring-like constructions occurred, obliterating the lumina of the vessels for only a small fraction of a

millimeter while the greater part of the veins remained widely open. As in the arteries, many of the constricted portions relaxed after a few seconds while new spasms appeared elsewhere, or, the original constrictions remained while the newer ones occurred, until the veins became segmented in appearance, or completely constricted. Occasionally widespread constriction of entire vessels took place at once.

The intensity of the reactions varied much from one batch of mice to another. By and large about 20 per cent of the sensitized animals showed almost complete obliterative spasm of all the ear arteries and veins for several minutes. In another 30 per cent spasm of the vessels was severe enough to produce complete obliteration of one or more arteries and veins in each ear, with partial or local obliteration of segments of many of the remaining vessels. About 17 per cent of the animals showed marked constrictions in arteries or veins but no obliterative spasm. About 10 per cent showed no constrictions, but instead stoppage or slowing of the circulation. The remainder, about 23 per cent, gave no visible reactions at all. No spasm or constriction of vessels was ever observed in the scores of control, unsensitized mice, injected with the same sera.

In severe shock, when complete obliterative spasm occurred in both veins and arteries, almost no blood remained in the blanched ears. In mild shock general obliteration of the arteries and veins was absent, and the local constrictions trapped the blood in the unconstricted segments, as has been mentioned above.

It is a matter of much interest that, in occasional instances the veins constricted before the arteries and in a few instances of mild shock spasms appeared in the veins while the arteries never showed constrictions at any time. The findings show that the venous constrictions were not brought about by a lack of blood in the vessels. Indeed, as will be seen below, all the constrictive changes took place while the carotid blood pressure stood either at the initial normal evel, or 10 to 40 mm. of mercury above it.

The Appearance of the Capillary Bed.—The appearance of the capillary bed differed much from animal to animal. To understand the differences it may be best, for descriptive purposes, to consider the vascular changes just described as falling into three general types; one in which arterial spasm set in before venous constriction; another, the commonest, in which arterial and venous spasm were synchronous; and finally, a relatively infrequent type characterized chiefly by venous spasm. Of course every possible combination of these types occurred, but as will be seen from what follows the happenings in the capillary bed were conditioned by the state of affairs in the larger vessels.

In most of the experiments in which there was obliterative spasm of the ear vessels the capillary bed blanched and the capillaries became invisible. This phenomenon occurred both in the instances in which arterial spasm preceded venous constriction and in those in which arterial and venous spasm occurred simultaneously. The capillary bed seemed to have emptied into the veins. An observer looking at the ear for the first time during this stage of shock might have readily assumed that an obliterative capillary contraction had occurred. However, this was not the case. In many experiments, one or two true capillaries (as defined by Chambers and his coworkers (20–23) were watched in one ear, at magnifications ranging from ×450 to ×900, while the changes in the larger vessels of the other ear were followed

through a low power (×80) microscope by another observer. When the capillary bed became blanched, as seen by the latter, the watcher at high power also lost sight of the capillaries he had been observing. Nevertheless, as he continued to search the spot where they had been visible, single blood cells suddenly appeared from time to time, passing rapidly through the invisible capillaries, which were obviously still patent but full of fluid. Presumably pressure from incompletely obstructed arteries skimmed off plasma which maintained a current flowing through the capillaries in which scattered blood cells moved on their way toward the veins.

When constriction of the veins occurred before that of the arteries, the true capillaries became choked with closely packed red blood cells. An observer seeing the ear at this stage for the first time might have remarked upon "capillary dilatation." However this is not the case; the capillary bed was simply pumped full with packed red cells.

It is clear from scores of such observations that the true capillaries of the mouse's ear are passive in anaphylactic shock; what happens in the capillary bed is determined by the site of spasm or constriction in the larger vessels. There is no contraction of the true capillaries, an observation which is in agreement with the recent findings of a number of workers studying other forms of shock (20–23).

The Recovery of the Circulation in the Ears.—In all mice that survived more than a few minutes, even in those that died after several hours and in those that, while surviving, yet showed complete obliterative spasm, recovery of the circulation in the ears began about 5½ to 20 minutes after beginning the serum injection. The first movement of blood took place either in the veins or in the arteries. In instances in which both the capillary and venous beds were blanched, the earliest movement of blood began in the arterioles and was followed at once by a prompt surge of the cells into the already patent capillaries. In a surprisingly short time all the vessels in the ear became filled with blood and widely distended. In the instances in which the capillary bed was already filled with cells, the direct arteriolar-venular channels (the A-V bridges of Chambers and Zweifach (20–23) opened first and blood passed through them into the venules, regardless of whether the latter were filled or empty. As result, in all instances the venules became pumped full of blood cells as recovery progressed, and true capillaries leading to the venules often became even more distended by a reverse flow. Very rarely one saw a plug of well packed cells forcing its way through a minute vessel. The ears soon appeared in the gross as though in a state of flaming hyperemia, but under the microscope one could plainly see that the movement of blood was excessively slow. As will be seen below the systemic blood pressure during this stage was low.

Figs. 1 to 6 illustrate, at low magnification (× 25), the changes that occurred in an ear during a moderately severe shock. The first photograph shows the ear before the serum injection, the second at one minute and a half after beginning it. Local obliterative spasms had occurred in practically all the vessels, trapping blood in them between the constricted segments. Fig. 3 shows the ear as it appeared 5 minutes after the injection. The arteries, together with many veins, had disappeared, but trapped blood can be seen in many other veins In this instance complete obliterative spasm did not occur, but the ear, save for the trapped blood, was blanched and white and the capillary bed was empty. Fig. 4 shows early recovery, 12½ minutes after the beginning of the injection. Veins here and there were filling with blood, but the arteries were scarcely

visible except under a higher power. Then one saw a hesitant, intermittent trickle of blood passing through them. Within 4 minutes more, however, ($16\frac{1}{2}$ minutes after the injection) all vessels in the ear had filled with blood (Fig. 5). The veins, distended almost to their initial calibers, were choked with closely packed, scarcely moving, blood cells. The capillary bed, too, had filled, but the arteries were still narrow and thread-like. Finally, half an hour after the injection (Fig. 6) the distended vessels gave to the ear the appearance of an intense hyperemia; but actually, for reasons to appear below, blood was scarcely moving through the organ.

In anticipation of findings to be given almost immediately below, it can be said that at the time that Figs. 2, 3, and 4 were taken, the carotid blood pressure was far above normal—and when the last two photographs were taken, it was far below normal.

THE BLOOD PRESSURE CHANGES AND THEIR RELATIONSHIP TO THE OTHER ANAPHYLACTIC PHENOMENA

It is generally believed that the clinical picture of anaphylactic shock results from some effect of the antigen-antibody reaction which leads, directly or indirectly, to constriction of smooth muscle in blood vessels, bronchioles, and other structures. A fall in blood pressure as a regular accompaniment of anaphylactic shock has been found in all animals in which suitable studies have been made. This has been ascribed by many to interference with the return of blood to the heart because of the constrictions of large veins in the abdomen or chest, a supposition substantiated by many *in vitro* experiments carried out with the Schultz-Dale technique and by the pathological findings in the lungs, liver, and other viscera in shocked animals. However in the later phases of anaphylactic shock there exists, along with low blood pressure, a profound vasodilatation. One would expect to find a compensatory vasoconstriction. Is the vasodilatation, therefore, an independent reaction, the result of the antigen-antibody reaction, or perhaps caused by nerve stimuli?

It is not known with certainty what rôle is played by the nervous system in the intact animal undergoing anaphylactic shock, nor what is the relationship between the vascular spasms, or subsequent dilatations, and the blood pressure changes. It seemed probable that the techniques for observing peripheral vascular changes here described, when combined with blood pressure measurement, might be used to answer some of these questions. We first determined what blood pressure changes, if any, occur in mice since this had not been previously studied. Next, after profound changes had been found, the time relationships between them and the onset of vasopasm and later dilatation were studied to learn which came first; that is to say, whether the vascular changes took place in compensation for the blood pressure changes or *vice versa*, whether they were occasioned by nervous stimuli, or whether they were independent reactions apparently brought about by the antigen-antibody reactions. The work has

shown that the vascular responses are independent of the blood pressure changes and furthermore are not determined by nervous stimuli.

Methods.—A previous paper from this laboratory (19) has described two methods for measuring blood pressure in the same mouse, one directly, by cannulation of the carotid artery, the other indirectly, by transillumination of the claws and observation of the blood flow in the claw bed, following inflation of a sphygmomanometer cuff placed about the thigh. Simultaneous measurements by both methods showed excellent agreement.

For the present work, mice, anesthetized with nembutal (19), were placed on their backs in the apparatus for measuring blood pressure (19), while their heads were supported in such a manner that the ears, with their dorsal surfaces facing downwards, spread out very lightly and without tension on glass slides. A few centimeters below the ears a mirror was fixed, and a strong cooled light, directed at an angle from below upwards, illuminated the dorsal surfaces of these organs, and allowed the brilliant reflexion in the mirror to be magnified by a binocular microscope. By these means the state of the blood vessels in the ear could be observed while blood pressure determinations were made at frequent intervals.

Thirty-two experiments were done. Eight normal mice were injected intravenously with 0.2 cc. of horse serum per 30 gm. of body weight. A second group of 16, sensitized to horse serum, were injected in the same way with similar amounts of the same serum. Of these, 11 showed spasms in the vessels of the ears. The remaining 8, sensitized to horse serum, were given intravenous injections of similar amounts of rabbit serum; that is to say, material containing proteins to which they had not been sensitized. In all, the systolic blood pressure was measured by both of the methods already mentioned (19) before, during, and after the serum injections, each of which required approximately 1 minute to complete.

Findings.—As already reported (19) the systolic blood pressure in the normal mouse anesthetized with nembutal or luminal, varies between 118 and 60 mm. of mercury depending largely upon the depth of the anesthesia. By the time that surgical anesthesia has been induced by the intraperitoneal injection of these anesthetics the systolic blood pressure is usually low, 60 to 90 mm. of mercury, and it may remain low for 15 to 45 minutes longer. As the anesthesia becomes lighter the pressure rises. Consequently in all the experiments to be considered here the pressure measurements were made at approximately the same time (45 minutes) after injecting the anesthetic. Only figures for the systolic pressure will be given hereafter.

Prior to the injections the systolic carotid blood pressure of all the mice ranged between 85 and 108 mm. of mercury. In some of them pricking the skin of the tail to inject the vein lowered the pressure by 5 to 8 mm. of mercury, but repeated prickings caused a rise of about 5 mm. Invariably the blood pressure was allowed to return to the previous level before the serum injections were begun. About 30 seconds after beginning the injections, when approximately half the dose had been given, the blood pressure began to rise by about 10 mm. of mercury. The rise continued to its maximum, usually 15 to 25 mm. above the preinjection level, in 1 to 3 minutes after the injection was completed. Occasionally the pressure rose by 30 to 40 mm. of mercury.

In the 8 normal mice, and in the 8 horse serum–sensitized ones injected with rabbit serum, there followed a gradual fall to the original pressure in the following 15 to 30 minutes. The blood vessels of the ears dilated slightly in some instances or remained unchanged while the circulation rate increased and the pulsation in the manometer in the carotid artery became greater.

Quite different were the findings in the 11 horse serum–sensitized mice which showed anaphylactic shock. In these the carotid blood pressure rose, as in the others, but at periods varying between 50 seconds and 3 minutes after the beginning of the injection, and either while the carotid blood pressure was still rising or while it remained at or near its maximum

height, spasm and constriction of the blood vessels of the ears took place. In several instances there was complete obliterative spasm of all the ear vessels and the organ was quite bloodless while the carotid pressure stood at levels 20 to 40 mm. of mercury higher than before the injection.

About 4 to 5 minutes after the beginning of the injection the blood pressure of the 11 mice that showed spasm of the ears began to fall while the spasm yet endured. Within 8 to 10 minutes the pressure usually reached the preinjection level, sooner than in the unsensitized mice or those receiving non-antigenic serum. One or 2 minutes later, when the pressure had reached a level about 20 mm. of mercury lower than the preinjection level, some of the ear vessels began to dilate and restoration of the circulation began. Thereafter the carotid blood pressure fell very rapidly, to 35 to 40 mm. of mercury, or even to 20 mm. or lower. The arteries and veins of the ears became more dilated, and the slow, stately flow of blood, already described, appeared in all of them. If death did not occur the blood pressure remained at levels between 20 to 45 mm. of mercury for an hour. Thereafter, further measurements by the direct method were abandoned.

Later Blood Pressure Changes. Studies of the Circulation in the Claws.—By the method for transillumination of the claws, already described (19), one observer watched the circulation in a claw bed of a hind toe, while another watched the mirror image of the ear vessels and noted the carotid blood pressure. The normal mice, injected with horse serum, and the horse serum–sensitized ones given rabbit serum, showed the usual rise in blood pressure, when the latter was measured in the leg, and also an increased circulation in the vessels of the claws. By contrast the circulation of the sensitized-shocked mice ceased at the same time that spasms began in the ear vessels, while the carotid blood pressure stood at the same high levels observed in the other animals which received serum injections, but in which there had been no blood vessel changes. The stoppage took place too rapidly to permit one to make pressure measurements by the indirect method. The blood cells in the claw bed remained immovable within the capillaries, which showed neither constrictions nor dilatations. When the circulation began again in the ears blood flow also reappeared in the claws of about half of the animals, indicating that in these instances spasm of the peripheral vessels in the ears and legs had relaxed simultaneously. In the remaining mice circulation in the claws reappeared later than in the ears, by periods ranging from 1 minute to 2 hours.

Since the blood flow in the claws ceased at the same time that vascular spasm occurred in the ears, the indirect method for measuring blood pressure in the legs was found useless during the acute stage of shock. However, animals with cannulated carotid arteries can be employed for limited periods only, and it was found possible to use the indirect method to advantage to study pressure changes occurring in the long period of recovery from shock. In 20 additional experiments blood pressure readings were made in the legs before shock was induced and again after shock, as soon as transillumination of the claws showed that blood flow had been resumed in the legs. The readings were repeated at intervals during the long period of recovery, or in the hours preceding delayed death, as the case might be.

When fatal shock occurred the blood pressure fell progressively to levels below 20 mm. of mercury and the animals died, either at once or within 1 to 3 hours. In the majority of survivors the pressure did not fall below 35 to 45 mm. of mercury, but even in these it required several hours to return to normal. The pressure of 4 of the 20 animals hovered between 18 and 30 mm. of mercury for 3 or 4 hours, the prostrated animals requiring no anesthetic or restraint while making the pressure measurements. Three of these died, but in the animal that survived the pressure began to rise slowly after the 3rd hour.

The findings show conclusively that the vasoconstriction in the ears and legs of the sensitized-shocked mice was not compensatory to a fall in blood pressure, since all the animals showed normal or elevated blood pressures at the time the

constrictions began. As shown by the experiments upon normal mice, part of the rise in blood pressure was due to the increase in blood volume occasioned by the injection of a relatively large amount of serum—0.1 cc. is equivalent to about 4 per cent of the blood volume of a 30 gm. mouse. The remainder of the rise in the shocked animals was conditioned, no doubt, by the closing off of many areas of the peripheral vascular bed by spasm of the arteries.

It is of interest that the carotid blood pressure began to fall while peripheral vasular spasm was still active, at least in the vessels of the ears. After the pressure had fallen to levels a little below the normal, vasodilatation took place, a reaction that can in no way be construed as compensatory in nature. It seems reasonable to assume that the vasodilatation was a primary reaction, and that it produced the fall in blood pressure. A vasodilatation occurring in the viscera or muscles could bring about the fall in carotid blood pressure even while the ear vessels remained in a constricted state. Work to be reported later has shown that such a vasodilatation does take place in the mouse.

THE PATTERN OF THE VASCULAR REACTIONS IS COMPLETELY INDEPENDENT OF THE BLOOD PRESSURE CHANGES AND OF NERVOUS CONTROL

Further experiments have shown more clearly that both the vasospasm and the subsequent vasodilatation of the ear vessels are wholly independent of the state of the blood pressure in the large arteries and that they are not brought about by nervous control.

It is generally believed that nerve stimuli are not of fundamental importance in the anaphylactic reaction. Seastone and Rosenblueth (24) have shown that the denervated nictating membrane of the cat contracts in anaphylactic shock. Lissak and Hodes (25) showed that anaphylactic shock of cats was similar, whether or not the sympathetic nervous system had been ablated, and Lissak and Kokas (26) found atropine to be without effect upon the progress of anaphylactic shock in dogs. These experiments, however, have ruled out special systems and specific mechanisms, not the nervous system as a whole, and additional evidence for or against the participation of the latter in anaphylactic shock is much to be desired. The techniques employed in the present work seemed to offer an excellent opportunity to throw light upon these questions.

Mice, sensitized and anesthetized as usual, were placed in plastaline moulds with only one ear lying on a porcelain placque. The other ear was spread horizontally over a smooth cork which had been selected and carved for each experiment until it fitted perfectly into the auditory meatus of the mouse to be studied. The corks were supported in the plastaline moulds, and the pinnae lay smoothly over them with result that the blood flow in the slightly curved upper surfaces of the ears could be observed through the binocular microscope in the usual manner. A rubber band, 3 mm. wide, attached to a scale pan, was slipped over the ear supported by the cork and adjusted close to the ear base. While observing the circulation in the vessels, weights were added to the scale pan until the arterial circulation and all movement of blood in the ear ceased. Usually about 22 to 28 gm., inclusive of the weight pan, sufficed.

The usual shocking dose of serum was next given to the animal, and the vascular reactions were watched in both the obstructed and unobstructed ears.

The findings from 12 experiments of this sort have been summarized in Table I, with the individual instances arranged from the top downwards in relation to the speed at which spasm appeared in the unobstructed ear following the injection of the antigen. Complete obliterative spasm of the ear vessels occurred in 5 of the 12 mice (4+ in column *b*). In two other animals approximately three-quarters of the vessels went into spasm (3+), in 3 others half of the vessels (2+) were constricted, more or less, and in one, less than one-quarter (1+).

TABLE I

The Speed of Onset and the Intensity of Anaphylactic Vascular Reactions in Ears with Free Circulation and in Ears Subjected to Temporary Circulatory Obstruction during the First Phase of Anaphylactic Shock

In ears with free circulation			In ears subjected to temporary circulatory obstruction				
(*a*)	(*b*)	(*c*)	(*d*)	(*e*)	(*f*)	(*g*)	(*h*)
Time to onset of vascular reactions*	Degree of spasm‡	Period of recovery§	Interval between recovery and release of block	Total period of block‖	Time to onset of spasm¶	Degree of spasm‡	Period of recovery**
min.		*min.*	*min.*	*min.*	*min.*		*min.*
0½	4+	10¾	6	18	0¾	4+	9¼
0¾	2+	11½	15	28	6½	2+	5½
0¾	4+	16	60	77	4½	4+	12
1⅓	3+	13	15	30	1	3+	4¾
1⅓	4+	10¾	24	36	0⅔	3+	14
1½	3+	16	8	25	8	2+	16
1½	4+	17½	25	40	0½	2+	14½
1¾	2+	7	19⅓	28	4	2+	12
2	2+	5½	5	12½	2	2+	5⅔
2	4+	10	3	14½	3¼	2+	14
3¼	1+	8	7½	16½	8	1+	17
One other animal showed no reaction in either ear.							

* After the beginning of the injection of antigenic serum.

‡ See text.

§ Time after the beginning of the injection, at which blood flow began again.

‖ These figures represent the total period of circulatory block from the time that the rubber band and the weights were placed over the ear until they were removed.

¶ After the return of blood to the ear.

** The time after the return of blood to the ear.

In one mouse there was no reaction. No correlation appeared between the speed of onset of spasm and the intensity of the reaction. In all these animals the spasm of the vessels relaxed in 5½ to 17½ minutes after the injection began and recovery of the blood flow took place (Column *c*).

In no instances were any changes noted in the caliber of the vessels of the obstructed ears. Had nervous impulses initiated the constrictions in the unobstructed ears there should have been spasm of vessels on the obstructed side, for the periods of circulatory stoppage at the times that the vascular spasms

occurred in the unobstructed ears were not long enough—less than 4 minutes—to block nerve impulses, nor was the mild pressure of the rubber band sufficient to do so.

Final proof that the vascular reactions were completely independent of the blood pressure changes, indeed that they probably provoked the latter, was obtained in the following manner.

At intervals ranging from 3 minutes to $1\frac{1}{4}$ hours after recovery of the blood flow in the unobstructed ears the rubber bands were removed from the obstructed ones. At once blood flow commenced and in every instance except No. 12 (Table I) in which there was no reaction in the unobstructed ear, spasm and constriction took place in the experimental ear after the remainder of the animal's body had passed through its shock reaction. As the table shows (column e) the onset of spasm after the first influx of blood in the experimental ear was sometimes quicker, sometimes slower than in the other ear, and the time required for recovery differed somewhat. The intensity of the spasm in the two ears of the same animal also differed slightly but by and large the reactions were like those observed in the unobstructed ear, during the initial shock. It was obviously a local shock reaction, a replica of the preceding one in the other ear.

It is important to note that, after release of the circulatory obstruction, vasoconstriction took place while the animals' blood pressure must have been very low, which was always the case when measurements were made in the mice during the recovery phase. By contrast, when the constrictions took place in the unobstructed ears the pressure must have been higher than normal. The inference is clear, that the pressure had no effect upon the occurrence of the vasoconstrictions.

DISCUSSION

The chief finding of the present work would seem to be the occurence of vasospasm and arrest of the circulation in the ears and feet of sensitized mice undergoing anaphylaxis. Such changes occurred not only while the blood pressure in the larger arteries was normal or slightly elevated, but at a time when unanesthetized animals, examined in the gross, showed either no symptoms whatever or at most only mild agitation and restlessness. The microscope revealed, both in unanesthetized and anesthetized animals, profound peripheral vascular effects imperceptible to the unaided eye. In the majority of cases the alteration was not fatal, and since it was to be perceived only by special methods, it can be termed occult anaphylaxis.

As already brought out, the constrictions and spasms of the ear vessels occurred only in sensitized mice, not in those previously normal. These reactions were primary phenomena in their own right and not dependent upon the changes in blood pressure or nervous stimuli. In mice poorly sensitized and showing little or no true shock at any time they were milder than in those

that passed later into severe or fatal shock, but the differences were of degree only, not of character. It is well known that sensitized or immunized mice yield notably poor skin reactions. This inference seems warranted, that the behavior of the ear vessels may be of service in the laboratory to detect slight degrees of anaphylactic sensitivity which might be missed by other means of investigation.

None of the earlier workers has studied the small vessels of the mouse during anaphylaxis. There has been no discussion of the reactions of minute peripheral vessels in sensitized animals not sufficiently hypersensitive to undergo actual anaphylactic shock. In two laboratories, however, constrictions and spasms of the vessels of the rabbit's ear have been observed during severe shock. Abell and Schenk (27) described them occurring within a Clark chamber in the animal's ear, and Bally (28–30) noted their appearance in the ears of rabbits during histamine, peptone, and anaphylactic shock. In this last and in histamine shock severe constrictions of the large arteries were seen early, about a minute and a half after the shocking injection, and after about 2 minutes, constriction of all the vessels took place. The blood pressure remained up until the spasms had reached their maximum, and fell thereafter, just as in our experiments on mice. By contrast, in peptone shock transient dilatation of the ear vessels occurred, followed by a late constriction after the blood pressure had fallen to its lowest point. The reactions witnessed by both groups of workers above mentioned must have been much like those which occur in the mouse.

Blanching in the ears of guinea pigs in anaphylactic shock has scarcely been mentioned in the literature, but a late cyanosis of the organs has been often reported. As is well known the systemic blood pressure first rises and then falls to low levels.

It would seem worthwhile to find out whether the ears of weakly sensitized rabbits and guinea pigs show peripheral vascular reactions in the absence of other signs of shock.

The present observations on mice showing severe shock have brought up some interesting possibilities. As already stated the behavior of the true capillaries of the ears was wholly unforeseen. They did not undergo any active constriction or dilatation, but simply remained patent and full of plasma though empty of red cells, or became crammed and distended with these latter, depending upon the state of affairs in the larger vessels. These findings fall in with those of recent workers (20–23) who have studied the behavior of true capillaries in other forms of shock.

The changes in the veins were pronounced, and the fact that they narrowed before the arteries did, or that in some instances constrictions occurred only in the veins, leads one to wonder whether something of the sort may not take place in skin during the development of wheals and other allergic skin phenomena in man.

It seems probable that the occurrence of occult anaphylaxis in generally sensitized animals showing no other signs of anaphylactic shock, and not locally sensitized, may have implications for the clinic. Allergists have long suspected that something like it happens in the sensitized tissues of man, and its occurence would explain instances of partial collapse, distress, anxiety, and other symptoms for which no cause has as yet been found.

SUMMARY

Pronounced vascular changes occurring in the ears and claws of mice during anaphylactic shock are described. Practically at once after a foreign serum (pig, horse, or rabbit) enters the blood stream of sensitized animals both the arterial and venous vessels undergo marked, local or generalized constriction in the organs mentioned. Usually spasm of the vessel walls occurs simultaneously in the arteries and veins, but it may appear first in the arteries, or occasionally in the veins. When venous spasm precedes arterial spasm, the true capillaries become distended with cells; if the reverse order holds, the ears appear bloodless. There is no active constriction or dilatation of capillaries; the capillary behavior follows passively the changes in the large vessels.

Peripheral vascular spasm occurs while the carotid blood pressure is high, but a few minutes later, while this still holds true, the ear vessels begin to relax and the circulation is resumed. Shortly afterwards the blood pressure falls to levels far below normal, but the vessels remain open.

If the circulation of one ear is obstructed while anaphylactic shock is produced, no vascular spasm occurs in it. Release of the obstruction during the animal's recovery results in belated constriction of the blood vessels of this ear although by now the vessels in the other ear are dilated and the general systolic blood pressure is very low.

The vascular reactions in the ears appear to be uninfluenced by the blood pressure in the large vessels, and they are not a response to nervous stimuli. They are local in origin.

The vascular changes are often not clearly perceptible in the gross but are plainly to be seen under a low power of the microscope. They occur in some sensitized mice exhibiting no manifest signs of shock, differing only in degree from the changes taking place when shock is severe or fatal.

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EXPLANATION OF PLATE 29

The photographs were made by Mr. Joseph B. Haulenbeek.

FIGS. 1 to 6. Vascular changes in the ear of a mouse during moderately severe anaphylactic shock. × 25.

FIG. 1. Before the shocking injection.

FIG. 2. 1½ minutes after the beginning of the injection. Local obliterative spasms had trapped blood between the constricted segments.

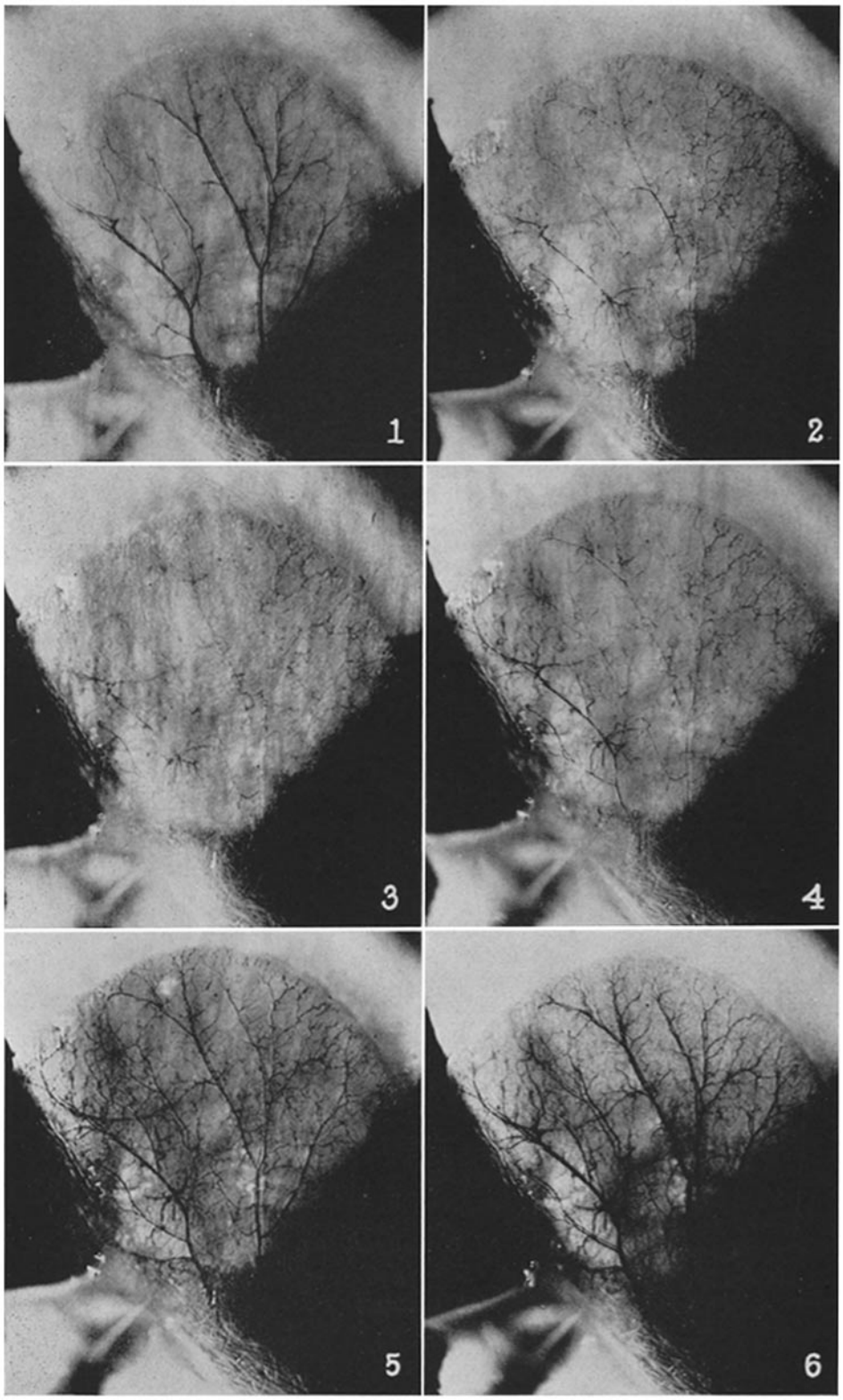
FIG. 3. 5 minutes after the injection. Most of the arteries and many veins had disappeared. In some veins trapped blood can be seen. The ear was blanched, the capillary bed empty.

FIG. 4. Early recovery, 12½ minutes after the injection. The veins were filling with blood which had previously begun to trickle through the scarcely visible arteries.

FIG. 5. 16½ minutes after the injection. All vessels of the ear were filled with slowly moving blood. The arteries were still narrow but the veins had reached their original calibers.

FIG. 6. Half an hour after the injection. The ear appeared intensely hyperemic, but the blood was scarcely moving in the vessels.

When the photographs for Figs. 2, 3, and 4 were taken, the carotid blood pressure must have been above normal—and when the last two photographs were taken it must have been far below normal.



(McMaster and Kruse: Vascular reactions in anaphylaxis)