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Influence of Intravascular Pressure on Vascular Response to Epinephrine.* (27753)

E. ASCHHEIM,† B. W. ZWEIFACH‡ AND M. B. ENGELBERG
Department of Pathology, New York University Medical Center, New York City

The increased peripheral resistance which accompanies the hypertensive syndrome has been ascribed to an increased reactivity of the smooth muscle elements of the terminal arterioles. In this context, the data have been interpreted as indicating the formation and systemic distribution of various vasoactive agents which either are vasoconstrictor by themselves(1,2), or potentiate the vascular response to adrenergic stimuli(3,4). Some workers believe that structural changes in the vessel wall can account for the heightened vasoconstrictor response(5,6,7), while others maintain that the distending force of the abnormal blood pressure places the smooth muscle elements under greater stretch, a factor which by itself may lead to a more forceful response to a given stimulus(8,9,10).

The procedures which have been used to investigate this aspect of the problem involve for the most part either plethysmography to assess changes in blood flow following intravascular infusion of vasoconstrictor agents (6,8,9,10), or the measurement of changes in tension of arterial strips exposed *in vitro* to a variety of experimental situations(5,7,11,12, 13). Although *in vitro* studies of isolated muscle preparations provide more quantitative data, information of this kind may be misleading, since it is tacitly assumed that conclusions based on changes in larger vessels are directly applicable to the resistance vessels. *In vivo* studies are difficult to interpret because of compensatory constrictor nervous influences. Perhaps the most direct approach to the problem under consideration would be to perfuse an isolated structure, such as the rabbit ear, at selected pressures so as to make it possible to relate the initial pressure and the flow decrement which is induced by vasoactive agents.

The perfusion data reported here show that epinephrine causes a greater reduction of flow at higher perfusion pressures when measured in absolute values, but that the per cent decrement of flow actually remains approximately constant.

Materials and methods. New Zealand albino rabbits (1500-2000 g body weight) were anesthetized with pentobarbital (35 mg/kg body weight), the ear removed with a sharp scalpel and the central artery cannulated. The ear was perfused with Tyrode's solution containing 1% (v/v) of citrated human plasma. Perfusion solutions were prepared a day earlier and filtered to avoid mechanical interference with flow because of the presence of small clots. Perfusion pressure was maintained at a given level by a suspended Mariotte's bottle. A water manometer inserted close to the cannulated ear indicated the perfusion pressure. The effluent was collected and weighed continuously on the pan of a suitable balance.

The isolated preparation was perfused at a constant pressure for 15 minutes to allow

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† Postdoctoral Fellow, U. S. Public Health Service.

‡ Investigatorship of Health Research Council of the City of New York.

for equilibration. Subsequently, 10 consecutive readings were made at one-minute intervals and their average taken as the flow per minute at this pressure. The vascular bed was then perfused with a mixture containing a given amount of epinephrine (10^{-3} μg/l) and the flow recorded by a similar procedure.

A total of 60 separate ears were studied. As will be seen in the data, the resistance of the ear vessels failed to return to the original control levels after epinephrine had been infused. It was therefore necessary to relate the actual decrement of flow induced by epinephrine to the flow during a control period immediately preceding it.

Regression coefficients were calculated, using the method of least squares(14) and regression lines then drawn.

Results. All of the flow measurements are compiled in Fig. 1. The pressure-flow values for separate experiments are listed without taking into account the previous status of the system. As the work progressed, it became obvious that vascular beds which were exposed repeatedly to epinephrine behaved differently from those which were stimulated for the first time *in vitro*. This point will be discussed later. When the regression lines are extrapolated to zero flow, they converge at a pressure close to 40 cm of water. This value may be considered to represent the critical closing pressure(15) of a single vessel equivalent to the perfused bed.

A plot was then made of the control flows in the above experiments, keeping those preparations which had been previously perfused with a test solution of epinephrine separate from runs in which the vascular bed had not been subjected to the constrictor action of epinephrine. As seen in Fig. 2, two regression lines can be drawn. The control values represent stabilized flows 15-30 minutes after a previous test perfusion, or in the case of untreated preparations after the same time interval of continuous perfusion. The data include only those preparations which were tested once with epinephrine. The differences between the two sets of regression coefficients are statistically significant.

The fact that the two lines follow a parallel course, indicates an identical rate of increase of flow with pressure for the two groups. By extrapolating the two regression lines back to zero flow, it is possible to demonstrate that vessels which had been previously perfused with epinephrine show a higher critical closing pressure.

In Fig. 3, the data are recalculated to express the decrease in flow following epinephrine relative to the pre-test control flow rate immediately prior to the epinephrine perfusion. The absolute decrease in flow is clearly a function of the perfusion pressure, being greatest at higher pressures. On the other hand, when the same data are expressed as per cent decrease in flow (Fig. 4), the value is independent of the perfusion pressure, being constant for a given concentration of epinephrine.

Discussion. The simple system used in the present study has several advantages. Unlike measurements made on living animals, the isolated microcirculatory bed of the ear is not subject to uncontrolled constrictor influences originating in the central nervous system. In addition, the effects noted cannot be attributed to possible structural modifications in the blood vessel wall, as might be the case in animals with established hypertension. The relatively constant nature of the system thus favored the assessment of the effects of a single factor, *viz.*, perfusion pressure on the effects of epinephrine vasoconstriction.

The higher critical closing pressure found in this study after exposure to epinephrine reflects an increased vascular resistance of the perfused bed. Along similar lines, an increase in the critical closing pressure (or its equivalent "flow cessation pressure") has been reported in intact animals coincident with an increase in vasomotor tone(16,17), an effect which can be counteracted by a diminution in sympathetic tone(16). The reason for the persistence of the higher critical closing pressure of ears following exposure to the constrictor action of epinephrine is unexplained. Repeated exposure to epinephrine has an even greater sensitizing action.

The present study emphasized the need to express pressure-flow data not in absolute terms, but as a fraction of the original value. Inasmuch as the intravascular pressure af-

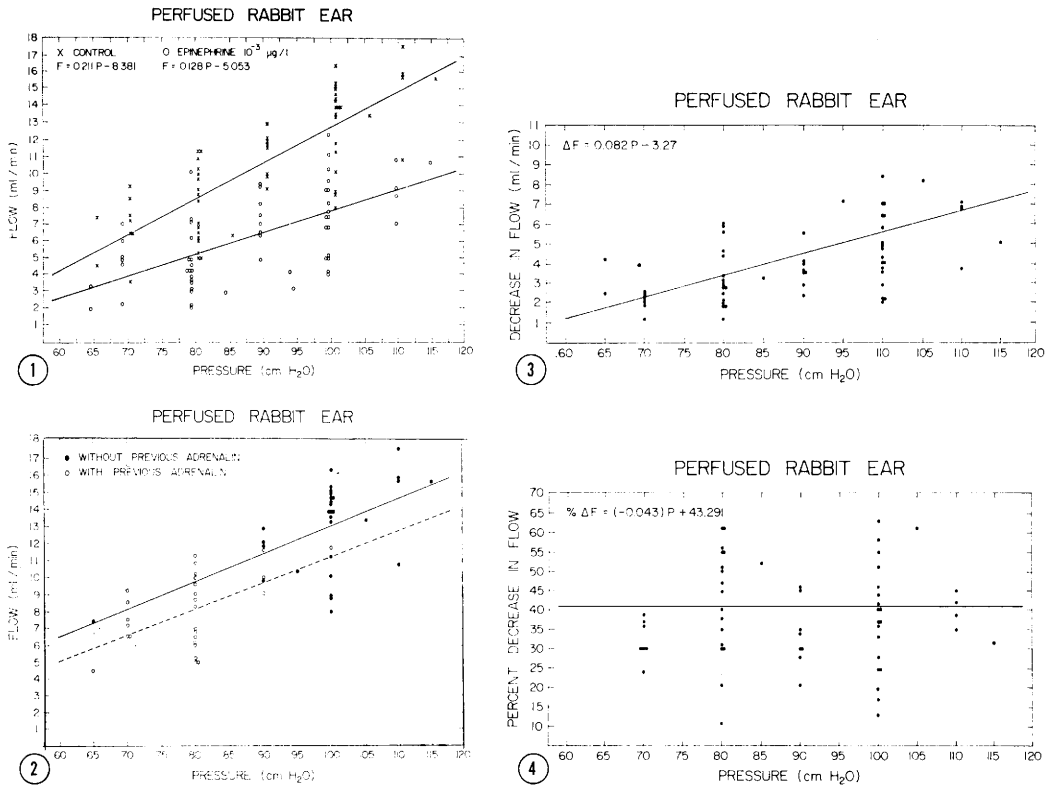

FIG. 1. Composite of 50 flow measurements at different perfusion pressures in isolated rabbit ear, comparing flow rates before and after a given dose (10^{-3} µg/l). Correction factor (see *Methods*) indicated in upper left corner.

FIG. 2. Stabilized flow rates at given pressure, separating those preparations (26) which had been exposed to 10^{-3} µg/l 15-30 min. previously from those (32) which were freshly prepared and had not been stimulated with epinephrine *in vitro*. Clearly indicated is a sensitizing action on the resting tone of the vascular bed. Pretreated ears exhibit a lower "closing pressure."

FIG. 3. Decrease in flow resulting from a given dose of epinephrine (10^{-3} µg/l) at different pressures.

FIG. 4. Effect of epinephrine on flow expressed as fraction of original flow level. The decrease in flow is clearly independent of the perfusion pressure.

fects the degree of stretch of the vascular smooth muscle, the results presented here lead to the same conclusion arrived at by workers using vascular strips *in vitro,* namely, that the tension developed by the vascular smooth muscle is a function of the initial stretch. This contingency must be considered relative to the conclusion reached on the basis of various plethysmographic investigations, *viz.*, that the greater decrement in flow induced by epinephrine in hypertensives as compared to normotensives indicates a difference in vascular sensitivity to catecholamines. A significant part of this effect may be due to the higher initial blood pressure of hypertensive subjects.

Summary. The vasoconstrictor effect of epinephrine on the isolated perfused rabbit ear was found to vary with the perfusion pressure. The drop in flow caused by a given dose of epinephrine was a constant fraction of the original value. This result suggests that the tension developed by the vascular smooth muscle depends on the initial stretch. It is concluded that the apparently greater effect produced by epinephrine infusions in hypertensives, as determined by plethysmographic methods, may be due in part to the higher initial blood pressure in these individuals.

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Lethality of a Forssman Antiserum for Mice.* (27754)

L. S. KIND AND J. DONCH
Department of Microbiology, University of California Medical Center, San Francisco

It has been known for many years that the tissues of the mouse contain the Forssman antigen(1), and recently the precise localization of this material has been visualized by the fluorescent antibody technic(2). The mouse, however, unlike the Forssman positive guinea pig, has not succumbed to the injection of Forssman antibody(3,4,5), and the absence of fatal reactions has been used to support the claim that mouse tissue does not contain true Forssman antigen(5). The following data indicate that pertussis-inoculated mice which have an increased susceptibility to the lethal effects of anaphylaxis, histamine, serotonin, etc.(6) can easily be killed with a Forssman antiserum.

Material and methods. A rabbit was injected subcutaneously once or twice a week for a month with boiled guinea pig kidney (Difco). Forty mg of the antigen was suspended in 1 ml of saline and the suspension mixed 1:1 with complete Freund's adjuvant (Difco) prior to injection. Ten days after the last inoculation the rabbit was bled and its serum was found to hemolyze sheep cells in the presence of complement (titer >5120). Portions of the rabbit antiserum to boiled guinea pig kidney (anti BGPK) were absorbed with one of the following antigens: (a) Boiled sheep erythrocyte stromata, a Forssman positive tissue (method of preparation described(7)); (b) boiled sheep erythrocyte stromata extracted with absolute methanol at room temperature, a procedure which removes the Forssman antigen(1); (c) desiccated boiled rabbit kidney which does not contain the Forssman antigen(1); (d) beef cell antigen (Difco), a heterogenetic antigen distinct from the Forssman antigen(8).

Aliquots of rabbit antiserum to BGPK (3.5 ml) were absorbed 4 times with 25 mg (per absorption) of one of the aforementioned antigens. Each absorption, lasting 5-10 minutes, was performed at room temperature and was accompanied by gentle stirring of the suspension. Centrifugations following absorptions were carried out at 2500 rpm for 10 minutes.

Swiss-Webster 20-25 g female mice sensitized intraperitoneally with 5 billion *B. pertussis* organisms killed wth 1:10,000 Thimerosol were injected intravenously 5-7 days later with 0.5 ml of absorbed or unabsorbed rabbit antiserum to BGPK. Deaths were noted within 1 hour although the great majority occurred 15-30 minutes after challenge.

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