Optical Density Measurements in Canine Tibias in Situ[1,2,3] (37256)

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(Introduction by E. J. VanLiere)

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Many investigators have made contributions to the field of photoelectric plethysmography since the pioneer work of A. B. Hertzman and associates in the 1930's and 40's. In their original articles, the Hertzman group reported applying the technique to various cutaneous vascular beds, as well as, the nasal septum (5). The original technique, however, utilized big, bulky, and relatively insensitive photoemissive cells. The development of photoconductive sensors made possible investigations of the optical density (OD) levels of vascular beds with small yet highly sensitive probes (4, 13).

The purpose of the investigation reported here was to extend the photoelectric plethysmograph technique to the measurement of blood OD changes in bone under controlled experimental conditions.

Methods. A detailed desciption of the instrumentation and of the experimental *in vitro* perfusion setup used in this study are published elsewhere (12). However, a brief description of the instrumentation is in order. A light source and a photocell with an 8000 Å filter were mounted in the opposite jaws of an adjustable clamp. A linearizing network was designed in which the electrical output of the system was a linear function of the optical density of the material placed between the light and the photocell, that is, between the jaws of the adjustable clamp.

For the *in vivo* experiments, adult mongrel dogs of varying ages weighing approximately 20 kg each were anesthetized with Pentobarbital (35 mg/kg). The right femoral artery and vein were cannulated to permit drug injections and perfusion of the limb with the animal's own blood, saline, or indocyanine green dye. Surgical exposure of the tibia was accomplished by means of an incision on the anterior midline of the leg, 3–4 cm below the knee. The fascia covering the anteriomedial aspect of the tibia was reflected from the bone. The posterio-lateral aspect of the tibia was exposed by reflecting back the tibialis anterior muscle. The adjustable clamp containing the photocell and light source was then firmly attached to the tibia. The entire limb was covered with a black rubber sheet to shield the preparation from changes in the background light levels. In order to facilitate the findings *in vivo,* and *in vitro* setup was constructed (12) which permitted saline, indocyanine green dye, and blood to be perfused through a lucite block in such a way as to permit OD measurements of these liquids flowing through a rigid section and a distensible section of 6-mm diameter (i.d.) tubing.

Results. Figure 1 shows an example of the OD changes seen when the limb was being perfused with the animal's own blood by means of a rotary pump and either acetylcholine or norepinephrine was injected into the femoral artery. Shortly after injection of 90 μg of acetylcholine (in 0.1 cc saline) a drop in the perfusion pressure to the limb occurs. This was accompanied by a sub-

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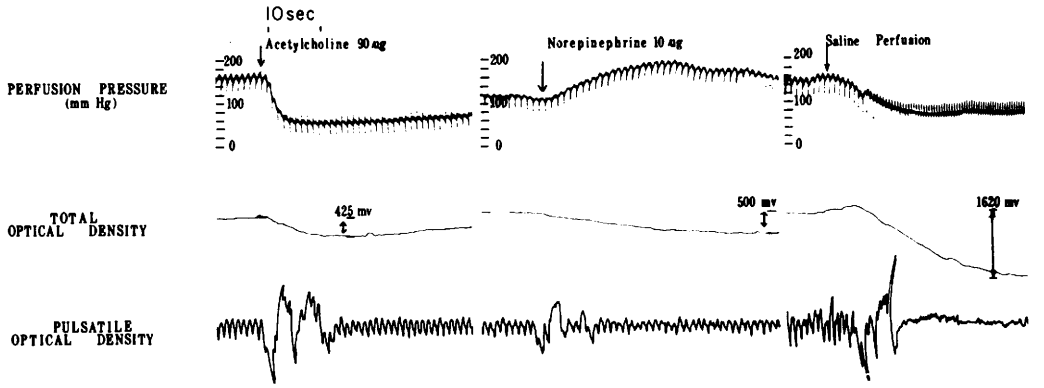

FIG. 1. Original recordings from a canine tibia giving results of acetylcholine and norepinephrine injections into the femoral artery and the control saline perfusion. The upper trace is perfusion pressure, middle trace is total OD, and lower trace is pulsatile OD. Both acetylcholine and norepinephrine caused significant decreases in the total OD while changing the perfusion pressures in opposite directions.

stantial decrease in the total OD level while the pulsatile OD level was essentially unchanged. The injection of up to 1.0 cc of saline alone did not noticeably alter the OD. When norepinephrine was injected, the perfusion pressure was seen to rise while there was again a drop in the total OD level and the pulsatile OD activity was minimally affected.

These maneuvers were followed in each animal by a period of saline perfusion. When saline was used as the perfusate, the total OD dropped to its lowest level and the pulsatile OD activity disappeared (Fig. 1). The time course of blood pressure and the total OD changes were plotted as percent of control using the change in OD during saline perfusion as the control (Fig. 2). The mean and standard errors are shown for 24 injections of acetylcholine and 26 injections of norepinephrine in 6 animals. The maximum decrease in the total OD seen with acetylcholine was 16%, reached in 20 sec from the beginning of the response, while the maximum total OD decrease seen with norepinephrine was 18% at 30 sec.

As shown in Fig. 1, when saline was used to perfuse the bone no pulsatile activity appeared while there was pulsatile activity when the bone was perfused with blood. In order to investigate this phenomenon further in an additional set of experiments, the limb was perfused successively with blood, saline, and dye. The pulsatile activity disappeared with saline perfusion as expected but then reappeared with dye perfusion. The percent

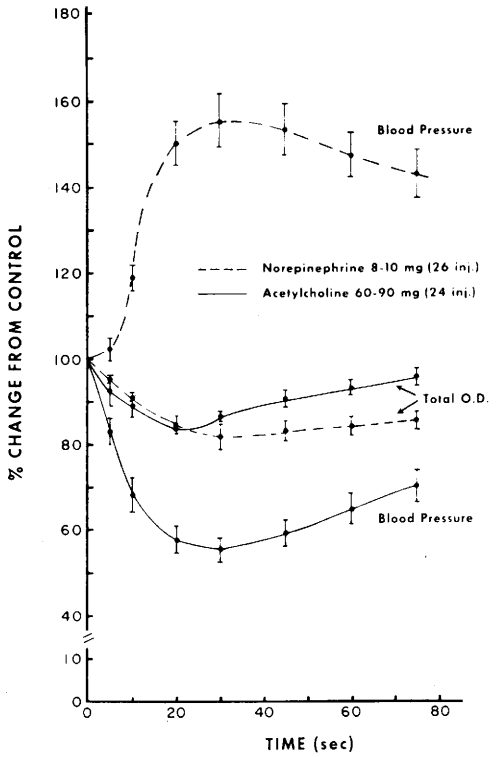

FIG. 2. Percent change from control of perfusion pressure and total OD for 24 injections of acetylcholine and 26 injections of norepinephrine. The means ± the SEM are shown.

pulsatile OD activity was calculated as equal to the pulsatile OD change $\times 100$ divided by the maximum OD change (seen during saline perfusion) since the output of our system is a linear function of the OD. In 13 trials on nine dogs the pulsatile OD while the limb was perfused with blood was $0.21 \pm 0.03\%$ of the maximum OD change. When the limb was perfused with dye the pulsatile OD activity was $0.15 \pm 0.04\%$.

Figure 3 is an original recording made from the rigid and distensible tubing perfused with saline, cardio-green dye, or blood. The results of this perfusion are very similar to the tubing perfusion published earlier (12). However, in addition to the blood and saline studies, a cardio-green dye perfusion was included in order to illustrate the use of dye to study volume changes. As shown on the top trace (Fig. 3a), the perfusion pressure remains constant throughout. During the perfusion of the rigid tube with saline the total OD was low while no pulsatile activity can be seen. The middle panel depicts the results seen when the rigid tube was perfused with dye. At this time the total OD is seen to assume a higher level while the pulsatile OD again does not reveal any activity despite a very high-gain setting. When blood is pumped through the rigid tubing, there is a total OD comparable to that seen with dye. In addition, however, there is now pulsatile OD activity occuring due to the presence of the red blood cells. Figure

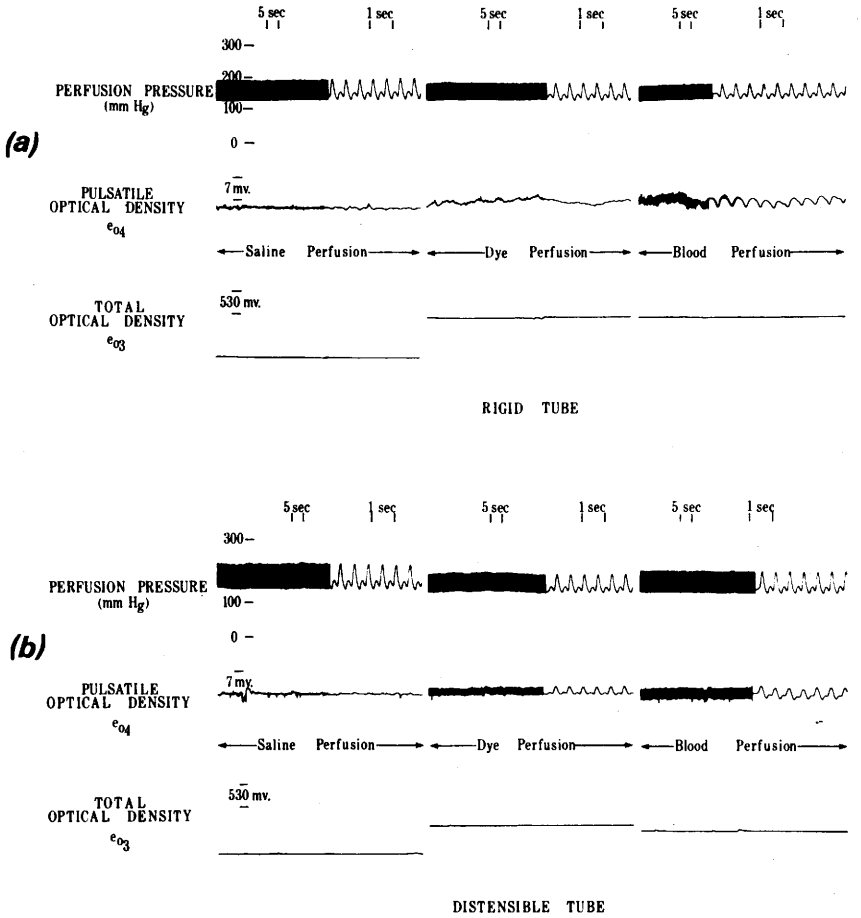

FIG. 3. Original recordings of perfusion pressure, pulsatile OD, and total OD for a rigid tube (a) and a distensible tube (b). Each trace begins with a saline control and is followed by dye and blood perfusion.

3b shows the results of perfusion of the distensible portion of the tube. Again, pressure is constant and with saline there is no pulsatile activity occuring. With dye, however, pulsatile activity occurs with the total OD at a comparable level to that of dye in a rigid tube. This pulsatile activity is the result of volume changes in the tubing since the flow-related phenomena are eliminated due to the absence of the red blood cells. With the perfusion of blood through the tubing, pulsatile activity also occurs, however. This pulsatile activity is different in waveform from that in the rigid tube with the higher-frequency volume-related pulses riding on top of the lower-frequency component due presumably to flow related phenomenon as shown in Fig. 3a.

Discussion. Large OD changes were seen when the vasoactive drugs were injected into the bone circulation. A possible explanation which will account for these changes of the total OD is an actual blood volume change within the bone marrow cavity which may behave as a semi-enclosed chamber. The evidence for bone acting as a semi-enclosed chamber stems from a series of medullary pressure studies (6–8). If bone is a semi-enclosed chamber, it is necessary to postulate the movement of fluid other than blood into and out of the medullary chamber as the blood volume decreases and increases over a period of time similar to Branemarks postulation of fluid shifts which might be necessary to account for intramedullary pressure changes seen after drug injections (2). Although interstitial fluid undoubtedly exists within the bone marrow, the lymphatic channels which would allow these rapid transfers have never been demonstrated.

Our findings in the case of norepinephrine are very much in accord with Branemark (2) who visually observed the decrease of blood flow in the marrow in a very similar total elapsed time of 1–2 min after norepinephrine injection. A decrease in blood flow is most likely responsible for the decrease in total OD seen with the injection of acetylcholine due to shunting of blood from the marrow to the extramedullary tissues of the limb. It is also possible that these occurrences can be explained on the basis of fluid shifts across the capillary membrane in accordance with Starling's considerations thereby accounting for blood volume changes in the face of a constant total fluid volume. This is, however, speculative and the consideration of this phenomenon as a possible cause awaits further investigation with pressure recordings from various areas of the capillary bed.

Accurate comparisons of small pulsatile OD changes were impossible where large OD level changes were present in the early photo plethysmographic studies (4, 10, 11, 13). No such difficulties are present with the system we have used since the output is a linear function of the input (12). The following possibilities exist as explanation for the pulsatile OD findings: (a) pulsatile blood volume changes; (b) flow-related phenomena (*i.e.*, red cell orientation, axial accumulation, and turbulent flow) which have been described by Bayliss and Taylor (1, 9); (c) oxygen saturation changes; (d) rouleaux formation [studied extensively by Brinkman *et al.* (3)]; (e) refractive index changes of the vessel wall secondary to geometric changes of the wall; and finally (f) the contrast phenomena seen when two media of differing extinction coefficients are mixing. The last five listed possibilities all seem to be of secondary importance since the pulsatile activity is present when the bone is perfused with indocyanine green dye. Thus, our results indicate that the majority of the pulsatile OD changes in bone are due to changes in bone blood volume.

If the 0.15% of the total OD seen when the bone was perfused with dye was accounted for completely by a vascular volume pulse, then 0.15% of the bone vascular volume would be changing with each pulse. By using the data collected by White *et al.* (14), in which the volume of blood per gram of tissue (27 cc/100 g) was measured by an isotope method in hypoxic limbs, we can calculate the actual maximum volume change necessary to account for the pulse. By assuming that the weight of the tibia of the dogs was 100 g, the volume change of approximately 42 μl per pulse per entire tibia is all that is needed. This amount of

fluid might be accounted for by that volume of interstitial fluid moving into and out of the various foramena in the bone with each pressure pulsation.

Summary and Conclusion. In 13 trials in 9 dogs, when the intact tibias were perfused by way of the femoral artery with blood and cardio-green dye at a constant rate (set to maintain equivalent blood pressure), the average pulsatile OD activity was 0.21 ± 0.03% and 0.15 ± 0.04%, respectively, when compared to the total OD change seen with the perfusion of the bone with saline. These results indicate that the majority of the pulsatile activity was due to volume changes within the bones' vascular system and not due to flow-related phenomena. When in six dogs, 24 injections of acetylcholine (60–90 μg) and 26 injections of norepinephrine (8–10 μg) were made into the femoral artery under constant flow conditions, the mean maximum total OD decreased 16% and 18%, respectively. These changes were most probably due to changes in the bone vascular volume.

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