EVALUATION OF LOCAL ISCHEMIA, GENERAL ANOXIA, AND VASODILATORS IN REACTIVE HYPEREMIA*

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Reactive hyperemia is a phenomenon characterized by an increase in blood flow to a tissue which has been temporarily deprived of its blood supply. There have been many attempts to explain the mechanism of reactive hyperemia. Bayliss[1] was the first to attribute the excess flow to physical changes which take place in the smooth muscle wall during the period of ischemia. He was able to show that the muscular coat of an artery wall reacts to decreased tension by relaxation. He further showed that this relaxation is a local event independent of nervous control or circulating hormones such as epinephrine. Folkow[2] observed that the blood vessels of the skin, leg and intestinal wall respond to a systematic reduction in blood pressure with a dilation. He concluded that these observations support Bayliss' original concept as to the physical nature of reactive hyperemia. Scott and Haddy,[3] Patterson,[4] and Geber and Schwinghamer[5] have also confirmed and extended the physical concept.

The other prominent explanation of reactive hyperemia was originally advanced by Lewis and Grant in 1925.[6] This hypothesis states that the elevated blood flow which occurs during reactive hyperemia is due to a vasodilation caused by anoxia during the period of occlusion. This anoxia is thought to produce vasodilation by acting directly on the smooth muscle of the walls, or more probably, indirectly by inducing the release of dilator metabolites.

The present investigation was designed to evaluate the relative role(s) of local ischemia, general body anoxia and vasodilator metabolites in the etiology of reactive hyperemia.

METHODS

All experiments were performed on dogs anesthetized with intravenous sodium pentobarbital (30 mg/kg). To prevent coagulation in the external blood flow circuits heparin sodium was administered in an initial dose of 5 mg/kg and in maintenance doses of 1.5 mg/kg every hour. A time lapse of 10 minutes was allowed following administration of either of these agents to minimize any untoward effects which might result from their action on the dog's vasculature.

The rate of flow in the femoral artery was measured with a simplified electromagnetic blood flow meter[7] and recorded on a Sanborn Twin-Viso recorder. Lateral pressure in the femoral artery, distal to the flow meter, was measured

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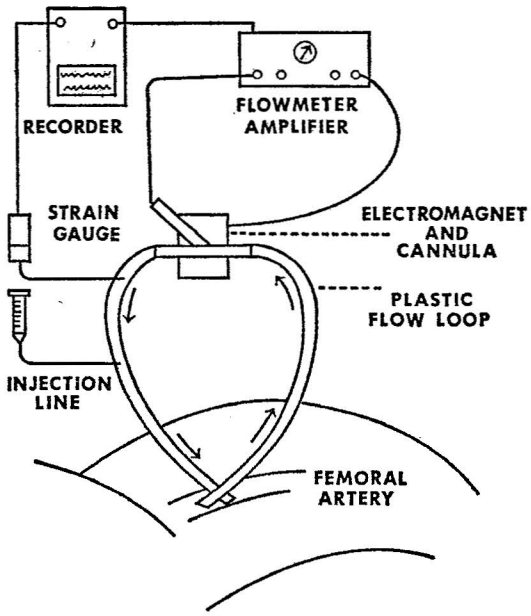

FIG. 1. Scheme of experimental setup illustrating relationship of flowmeter and strain gauge to femoral artery.

with a Statham strain guage P23AA. Figure 1 illustrates the position of the strain gauge and flow meter. Blood flow through the femoral artery was stopped by clamping the loop.

A modification of the experimental setup utilized in five dogs was the isolated leg preparation. By this method the hind leg was dissected free of the body with the exception of the femoral artery, femoral vein and sciatic and saphenous nerves which remained intact. A series of experimental manipulations was made with intact nerves and one series following nerve section. No demonstrable differences were noted between the two types of preparations. The isolated leg was therefore deleted from further study in the present investigation.

All chemical compounds were prepared 30 minutes before use by dilution with mammalian saline and warmed to body temperature before injection.

Short duration occlusions. The first procedure in each experiment consisted of a series of occlusions having the following durations: 3, 5, 8, 10, 15, 30, 45, 60, 120 and 180 seconds. Special attention was given to the hyperemic response which followed the very short periods of ischemia.

Venous, arterial-venous occlusion. It was felt that the usual method of inducing reactive hyperemia by arterial occlusion alone might allow a potential dilator substance being produced in the occluded area to be carried away by venous drainage. To hold any such compound in the vasculature upon which it would react the following manipulations were carried out: (1)

simultaneous arterial-venous occlusion; (2) arterial occlusion followed by venous occlusion; (3) venous occlusion followed by arterial occlusion; (4) venus occlusion alone.

Dilator substances. Attempts were made to determine the possible role several different dilator substances might have in the genesis of reactive hyperemia. The first compound studied was histamine. The dog's vascular system was rendered insensitive to histamine by injections of 300 mg of either diphenhydramine (Benadryl) or tripelennamine (Pyribenzamine), both of which are known to be potent antihistaminic agents. When an amount of histamine, *i.e.*, 0.1 mg, which formerly produced a marked dilation was rendered ineffective, or nearly so, by the antihistamine injections, a series of arterial occlusions was carried out and the hyperemic responses were compared with control responses taken prior to the antihistamine injections.

Serotonin has been shown to be capable of producing vasodilation. Therefore, it seemed reasonable to examine its possible role in hyperemia. Antiserotonin compounds, lysergic acid diethylamide tartrate (LSD, 0.1 mg), 1-methylmethergine tartrate (UML, 0.1 mg and lysergic acid diethylamide bitratrate (BOL, 0.1 mg) were injected directly into the femoral circulation. Following the injection of these serotonin antagonists a series of arterial occlusions was carried out and the hyperemic responses were compared with control responses.

Lactic acid is another biologically occurring compound which has the ability to produce vasodilation. To investigate the possible role which lactic acid might have in reactive hyperemia the following experiment was performed. Solutions of unbuffered lactic acid varying in concentration from 0.01 per cent to 3.0 per cent were injected directly into the femoral circulation and the effect on resting femoral blood flow was noted.

Prolonged contralateral occlusions. Two techniques were employed in this phase of the study. The first consisted of occlusion of the femoral artery in the leg not being monitored for blood flow and pressure. Periods of occlusion ranged from 3 to 30 minutes during which time the flow and pressure were continually recorded from the opposite femoral artery. Following release of the occlusion the flow and pressure recordings were evaluated for an additional 30 to 45 minutes to allow for possible latent or delayed reactions. The second procedure was a variation on the first type. It consisted of monitoring brachial artery blood flow and pressure before, during, and after occlusion of either one or both femoral arteries.

Progressive decrease in respiration rate and reactive hyperemia. To examine the possible role which total body anoxia might play in reactive hyperemia, the following procedure was carried out on nine dogs. The chest cavity was opened producing a pneumothorax. The animals were subsequently maintained on artificial respiration at 16 to 20 cycles per minute with a Harvard Respiration Pump. A series of occlusions of 15, 30, 45, 60, 120 and 180 seconds was made while the dog was being respired at this rate. The respiration rate was

then decreased to 10 cycles per minute. The animal was maintained at this new level for 5 minutes, following which the above series of occlusions was repeated and the hyperemic responses noted. A second series of occlusions was repeated at 10 minutes following the first series. The total procedure was now repeated at a respiration rate of 5 and 2 per minute. Finally, the respiration rate was reduced to zero. The dog was allowed to remain in the apneic state for 2 minutes; at the end of this time occlusions of 30 and 60 seconds were produced.

Nitrogen inhalation anoxia and reactive hyperemia. To examine the role of oxygen lack in another manner, the following procedure was undertaken: animals were artifically respired at 16 to 20 cycles per minute as described above and then were allowed to breathe "pure" nitrogen (99.2%) for 2 minutes. At the end of this 2-minute period, the femoral loop was clamped for 15 and 30 seconds and the responses which followed release of these occlusions was observed and recorded.

RESULTS

Short duration occlusion. It was noted that occlusions as short as 3 seconds were usually capable of producing a hyperemic response as shown in figure 2. The magnitude of the response increased with the duration of occlusion for periods up to 30 to 180 seconds. Beyond this time there was not always a correlation between length of occlusion and degree of response. In some ani-

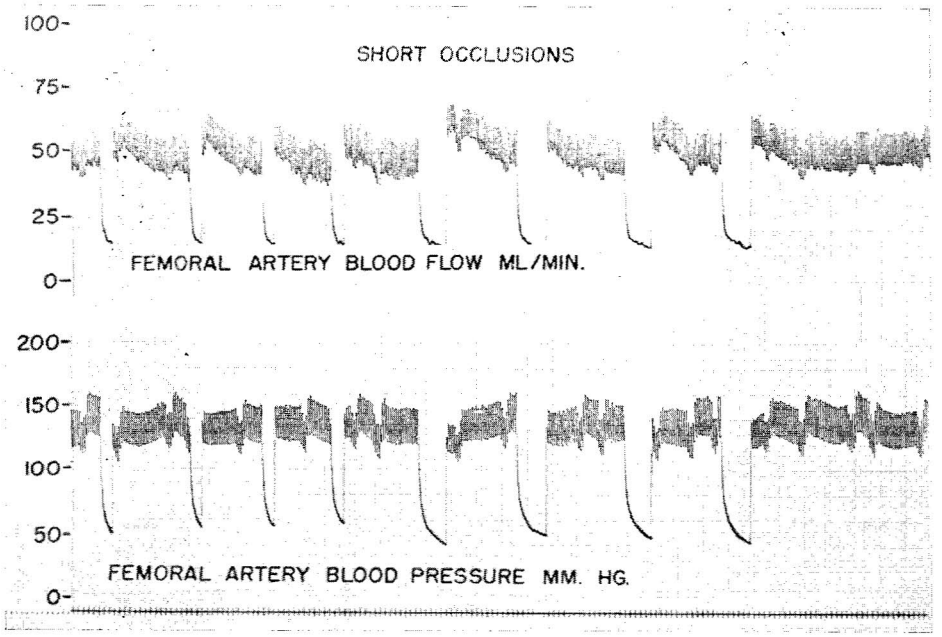

FIG. 2. Note the well defined reactive hyperemic response with arterial occlusions ranging from 2 to 6 sec.

mals the maximal response appeared to be reached following a 30-second cessation of blood flow and longer periods of ischemia did not result in a greater hyperemia. In other animals the maximal response was not reached until the duration of occlusion was much longer, i.e., about 150 and 180 seconds. The average occlusion time which produced maximal hyperemia for all the dogs included in this study, was found to be 55 seconds ±20 seconds. The increased flow seen during hyperemia was usually accompanied by a concomitant fall in blood pressure. There usually existed a rough correlation between the degree of flow increase and pressure decrease, however, several instances occurred where an animal showed the ability to increase flow while maintaining pressure relatively stable.

Venous occlusion. This procedure was carried out on 10 dogs. In eight of these animals occlusion of the femoral vein produced no hyperemia response, in the other two animals there was a slight elevation in blood flow following longer periods of occlusion; in both of these cases this elevation was much less than that produced by an arterial occlusion of similar duration and was, in fact, small enough to be considered negligible.

Simultaneous arterial and venous occlusion. This procedure was carried out on six dogs. In every case the magnitude of the hyperemic response produced by simultaneously occluding the femoral artery and vein was much less than that produced by an arterial occlusion of the same duration. Figure 3 illustrates this observation.

Dilator substances. The antihistaminic drug experiments were carried out on four dogs using Benadryl and on two dogs using Pyribenzamine. Dosage levels of those substances which were capable of rendering the dog's vasculature relatively insensitive to injected histamine did not change the character, magnitude or duration of the hyperemia response.

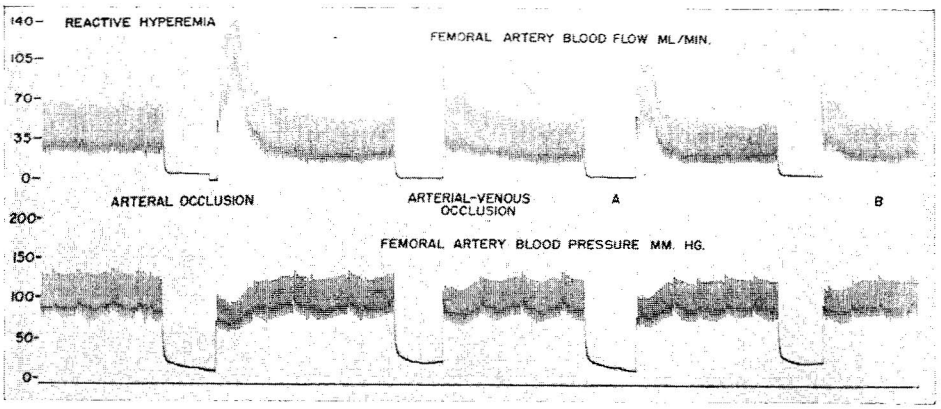

FIG. 3. Note the almost total absence of reactive hyperemia with a 15 sec. arterial-venous occlusion and the greatly reduced response to a 30 sec. occlusion of the same type. In contrast the arterial occlusions alone produced marked reactive hyperemia.

The procedure employing serotonin antagonists were performed on four animals. In each case it was found that injection of 0.1 mg of serotonin directly into the femoral loop resulted in a decrease in blood flow. This, in itself, would seem to indicate that serotonin is not involved in reactive hyperemia. Furthermore, it was observed that none of the serotonin antagonists altered the hyperemic response to any extent.

The lactic acid experiments were carried out on three dogs. There was never any hemodynamic response to lactic acid infusion until the concentration reached 3.0 per cent, and here it required 0.6 cc to cause a very small dilation.

Prolonged contralateral occlusions. None of the 12 dogs studied showed any elevation in flow through the right femoral artery following removal of arterial occlusion and restoration of circulation through the contralateral vessel. A similar lack of flow change was seen in those experiments where flow was measured in the forearm.

Respiration rate variation. In every case it was observed that neither progressive reduction in respiration rate nor complete cessation of breathing increased the hyperemic response; in several cases the responses were actually decreased. Figure 4 illustrates the effect of respiratory arrest on reactive hyperemia.

Nitrogen inhalation. The inhalation of "pure" nitrogen caused the blood flow and pressure to rise rather rapidly and reach a plateau within 1 to 2 minutes. Both flow and pressure were maintained at these levels for periods of from 1 to 2½ minutes. Figure 5 indicates that the profound anoxia induced by this method did not increase the hyperemic responses. Actually the response was decreased both in amplitude and duration in many instances. Note, however, that acetylcholine was still capable of increasing flow when injected into the blood flow circuit.

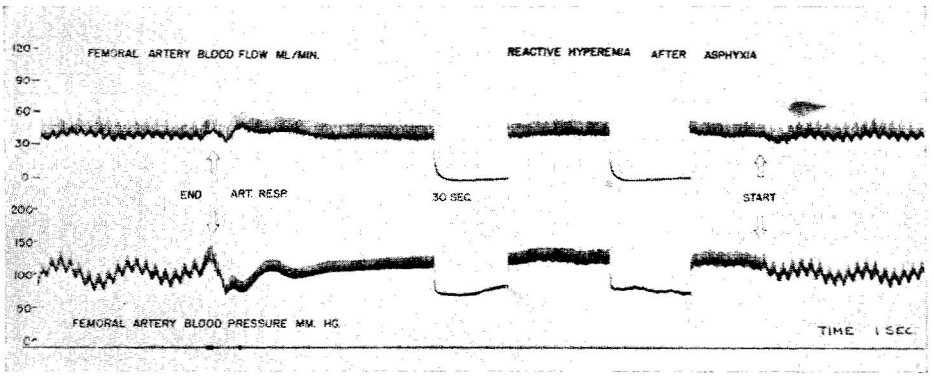

FIG. 4. Dog artificially respired with air. At *End Resp.* on record respiration pump was stopped. Note 60 sec. of arterial occlusion did not produce reactive hyperemia following 65 sec. of respiratory arrest.

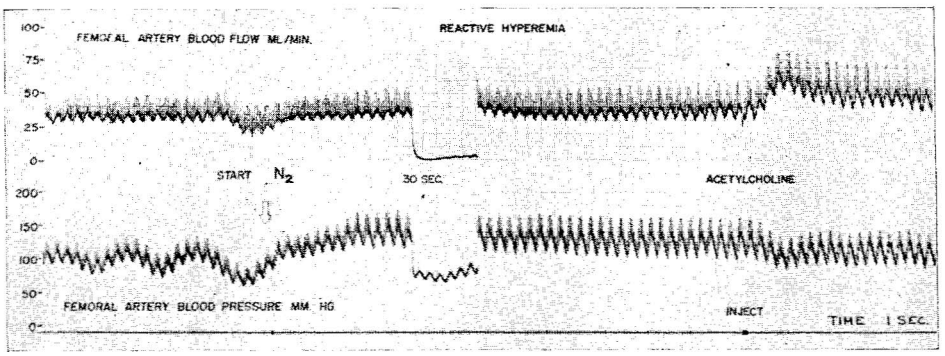

FIG. 5. Dog artificially respired with air. At *Start* N_2 on record animal was switched to pure nitrogen gas. Note virtual absence of reactive hyperemia following 60 sec. of breathing this gas. Also note that acetylcholine (10 μg.) injected into the femoral artery elicits a well defined flow and pressure response.

Observation of the color of the blood flowing in the plastic tubing in the external flow loops during both nitrogen inhalation and low respiration rates indicated extremely low oxygen tension.

DISCUSSION

The present observation that occlusions of very short duration can repetitively produce hyperemia is consistent with the original findings of Bayliss. This fact seems to indicate that the primary cause of reactive hyperemia is not the liberation of a vasodilator(s) during periods of induced ischemia since it would appear virtually impossible for an occlusion of such short duration to produce anoxia sufficient to result in the release of dilator substances in amounts necessary to cause the observed increase in blood flow.

Venous, arterial venous occlusion. The inability of venous occlusions to produce hyperemia confirms the findings of Hilton[8] and seems to cast further doubt on the chemical explanation of reactive hyperemia, since, if the cause of this event was the release of a dilator substance, every procedure which produces an ischemia should result in a period of hyperemia. Thus, venous occlusion should result in an anoxic state approximately equal to that produced by arterial occlusion. In addition, it was reasoned that if reactive hyperemia is due to the release of dilator metabolites during ischemia then it should be possible to enhance the effect of these metabolites by occluding the major venous drainage of an area, thereby tending to trap the dilator compounds in the vasculature and giving more opportunity to exert an effect. This enhanced effect should then be reflected as a greater hyperemia. The results of these experiments indicate that the hyperemia produced by arterial occlusion alone is of greater magnitude than that produced by simultaneous arterial and venous occlusion. This observation, coupled with the inability of venous occlusion by itself to elicit a hyperemic response, appears to cast further doubt on the chemical origin theory of reactive hyperemia.

Dilator substances. The antihistaminic drugs investigated were incapable of altering the hyperemic response to arterial occlusions of 130 to 180 seconds. This finding agrees with the observations of several other workers who have employed a number of different techniques of blood flow and pressure measurement.[9, 10] Since these agents have been shown to be capable of rendering an animals' vasculature insensitive to injected histamine, the simplest conclusion is that histamine is not primarily involved in reactive hyperemia. There is, however, an alternate possibility which has been proposed by Dale[11] who claims that the fact that antihistaminic drugs do not alter the hyperemic response can not be taken as absolute proof that histamine is not involved in this phenomenon. He has introduced the concept of "intrinsic histamine" which he explains by pointing out that the cells which release histamine, and those which respond to it may be identical. If this situation exists, then antihistamine introduced into the blood stream might be relatively ineffective. Dale offers no data to support this theory and there are several facts which seem to oppose his views. First, there is the observation of Emmelin and Emmelin[12] who point out that intravenous antihistamine is capable of diminishing the response of the skin vessels to intracutaneously injected histamine. Second, there is the argument of Landowne and Thompson[13] who note that antihistaminic agents probably act via their competitive or combining powers at the site of action of histamine. They say that this site of action must be either on the cell surface or within the cell, and since antihistaminic drugs inhibit the action of exogenous histamine, they must have reached this site of action. Thus, it would seem that Dale's "intrinsic histamine," whether it acts outside or within the blood vessel cells responsible for the dilation seen in reactive hyperemia, should be inhibited by circulating antihistamines. The above quoted observations of other investigators, coupled with the present work, would seem to firmly establish the fact that histamine can not be considered a primary causitive factor in reactive hyperemia.

The present study with serotonin, and with several drugs known to antagonize its action, has led to the conclusion that this compound is, in all probability, not involved in reactive hyperemia. This is based on two observations. First, the finding that serotonin injected directly into the femoral circulation results in a decreased blood flow. This indicated that at least in these particular dogs the compound was acting as a vasoconstrictor and hence could hardly be expected to cause the increased blood flow seen during the hyperemic response. Second, the fact that large doses of several drugs known to antagonize the action of serotonin did not alter the hyperemic response can be taken as an indication that serotonin probably does not play a prominent role in reactive hyperemia.

Additional experiments indicated that relatively large amounts of lactic acid are required to produce even a very small increase in blood flow. It would also seem that occlusions as short as 3 seconds are incapable of causing an ischemia great enough to result in the production or release of this much

lactic acid. These facts indicate that lactic acid is probably not involved in the genesis of reactive hyperemia.

Contralateral femoral and brachial artery occlusion. These manipulations were suggested by the work of Freeburg and Hyman[14] who obtained plethysmographic measurements of forearm blood flow in humans and reported an increase in the rate of forearm flow following a period of arterial occlusion in the leg. They attributed this increase to a blood borne vasodilator substance from the ischemic leg. This observation would appear to indicate that vasodilator substances are indeed liberated during ischemia and are probably mainly responsible for the increased flow seen during reactive hyperemia. The first series of experiments reported in this paper in which blood flow in one femoral artery was measured during and after occlusion of the contralateral vessel fails to confirm the findings of Freeburg and Hyman. In an effort to more closely duplicate their procedure, a second series of experiments was undertaken in which flow was measured in the brachial artery before, during and following femoral arterial occlusion. The results of this second investigation support those of the first study in failing to confirm the findings of Freeburg and Hyman. This incongruity may be due in part to differences in subjects or techniques of blood flow measurements. The experiments of Freeburg and Hyman involved plethysmographic measurements of forearm flow in human subjects while the present study consisted of direct electromagnetic flow measurements in femoral and brachial arteries of dogs.

Respiration rate reduction, nitrogen inhalation. Both the respiration rate reduction and nitrogen inhalation experiments indicate that oxygen lack is not the prime cause of reactive hyperemia. If reactive hyperemia was due primarily to oxygen lack, then lowering the oxygen saturation of the blood should result in a greater hyperemic response since each occlusion would begin in, and would be followed by, a condition of comparative hypoxia rather than a fully oxygenated state.

These observations are supported by the findings of several other investigators. Dornhorst and Whelan[15] were able to show that arterial hypoxia induced by inhalation of an 8 per cent oxygen 92 per cent nitrogen mixture during 2 minutes of a 20-minute ischemic period and during the recovery period had little effect on the duration of the increased blood flow which followed release of arterial occlusion. Here the blood oxygen saturation had been greatly reduced, therefore, it should have required a much more prolonged elevation in flow to repay the so-called "oxygen debt." Since this was not the case, one is led to conclude that the work of Dornhorst and Whelan sheds further doubt on the anoxia theory of reactive hyperemia. The work of McNeil[16] also seems counter to the anoxia hypothesis. He found that during reactive hyperemia the oxygen saturation of the efferent venous blood rises well above the previous resting level; from this he concluded that hypoxia is unlikely to be a cause of reactive hyperemia. Thus, the findings of these investigators coupled with the present observations indicate that a decreased

oxygen tension is not the paramount factor involved in the causation of reactive hyperemia.

Crawford *et al.*[17] and Ross *et al.*[18] have shown that progressive reduction in the oxygen saturation of blood perfusing the hindlimb of a dog results in a concomitant increase in blood flow to this area. This observation had led them to conclude that oxygen lack might well be the cause of reactive hyperemia. It should be pointed out, however, that these studies involved only a reduction in oxygen saturation, and in neither study was the blood flow actually occluded. Since reactive hyperemia has repetitively been defined as the increase in blood flow which follows circulatory arrest,[4, 8, 16, 18, 20] and since these investigators never produced such an arrest, it would seem that their work is not sufficiently analogous to warrant this conclusion.

SUMMARY

The relationship of local ischemia, general anoxia and vasodilator metabolites to the production of reactive hyperemia has been investigated utilizing the vasculature of the hind limb of the dog.

The oxygen tension of the circulating blood was reduced to extremely low values by the techniques of (1) progressive reduction of respiration rates down to zero, and (2) inhalation of pure nitrogen at normal respiration rates. In no instance was the reactive hyperemia influenced by these conditions.

Very short occlusive periods, i.e., 3 seconds, produced hyperemic responses. It is doubtful if this short occlusion could produce significant concentrations of known vasodilator metabolites or reduction in oxygen tension in the occluded vasculature areas.

Venous occlusion, in contrast to arterial occlusion, does not produce a hyperemic response. When a venous occlusion precedes or is made simultaneous with an arterial occlusion, the resulting hyperemia is much less than with arterial occlusion alone.

Relatively large amounts of lactic acid injected directly into the femoral circulation were required to produce a relatively small increase in blood flow.

Compounds known to antagonize the actions of histamine and serotonin were ineffective in altering the hyperemic response.

ACKNOWLEDGMENT

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