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Hesperidin, Phagocytosis, and O_2 and CO_2 Tensions in Subcutaneous Gas Pockets.* (28109)

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Gas from artificially-induced subcutaneous pockets in unanesthetized rats can be used to study the relation of local blood flow to local metabolism of the subcutaneous tissue(1). Actions of various agents in a circumscribed region can be observed by administration of drugs or chemicals directly into these pockets.

Pocket tensions of O_2 and CO_2 reflect tissue tensions, and are expected to change in a reciprocal manner—high local blood flow increases pO_2 at the same time that it decreases pCO_2; low blood flow or high aerobic metabolism decreases pO_2 while increasing pCO_2. However, it is shown below that when the flavonoid hesperidin was injected into pockets, CO_2 increased but O_2 did not uniformly decrease; many times both pO_2 and pCO_2 were higher than in saline-injected control pockets. To investigate some of the explanations which occurred to us, we injected particulate substances, acid, and a vasoconstrictor agent into pockets.

Methods. Pockets were formed in the subcutaneous tissue on the backs of female rats (200 g, NMRI Long-Evans strain) by injection of 20 ml of wet air with hypodermic needle and syringe(1). Additional injections every 3 or 4 days replaced gas absorbed into the tissues. Samples of pocket gas aspirated into syringes were analyzed for O_2 and CO_2 (2). No attempt was made to use sterile technic, and there were no indications of infection in the rats or their pockets. Some rats were sacrificed and tissue from the gas pocket wall was fixed for histological examination.

Preparations injected into the pockets were phosphorylated hesperidin, 10 mg/ml; commercial India ink, 0.1 ml/ml; trypan blue, 10 mg/ml; epinephrine, 1/50,000; and lactic acid, 5%. The solvent or suspending medium was saline in each case except for lactic acid when water was used. Pure saline served as a control injection.

Results. Each of the 61 points in Fig. 1 represents the pO_2 and pCO_2 of a single analysis of pocket gas in one of 33 rats receiving an intrapocket injection of 1 ml per day of the phosphorylated hesperidin preparation during a 5 day period. The shaded box contains 95% of 68 samples obtained from 28 saline-injected or untreated controls. (The black rectangle is mean of the control samples, the dotted line a regression line of pCO_2 on pO_2, the oblique boundaries are 2 SE from the regression line, and the vertical boundaries are range for the pO_2 values.) Most of the points with both O_2 and CO_2 above the control mean occurred after 3 days of treatment.

Fig. 2 shows samples obtained from 12 rats, 3 rats per treatment, during a 3 day pe-

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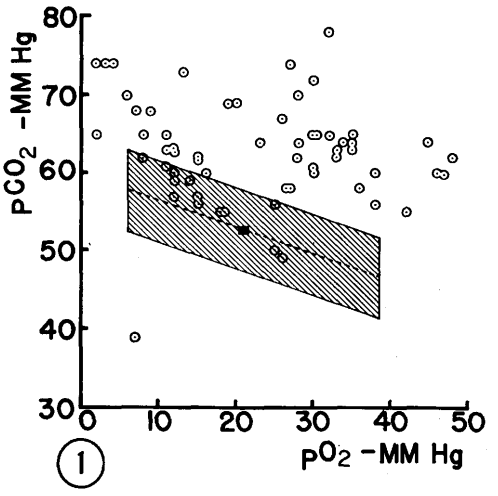

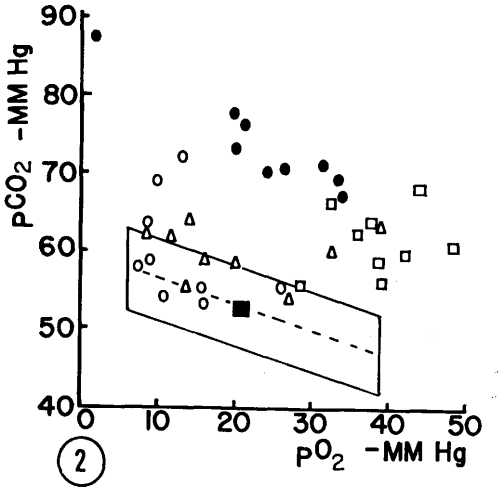

FIG. 1. Effect of intrapocket injections of hesperidin on gas tensions in subcutaneous pockets in rats; pCO_2 plotted *vs* pO_2. Ninety-five per cent of 68 samples from untreated or saline-injected controls lie within the shaded box.

FIG. 2. Effect of intrapocket injections of particulate substances and hesperidin on gas tensions in subcutaneous pockets. Box of control values same as in Fig. 1, ○ = hesperidin, □ = India ink, △ = trypan blue, ● = hesperidin plus India ink.

riod. Treatments were 1 ml/day intrapocket injection of (a) India ink, (b) trypan blue, (c) hesperidin, or (d) India ink plus hesperidin (1 ml/day of both). The control box is the same as in Fig. 1. Each treatment caused the phenomenon of elevated pCO_2 associated with elevated or normal pO_2 at least part of the time during the short treatment series.

In 3 rats, repeated intrapocket injections of ½ ml of the epinephrine preparation every 5 or 10 minutes (to simulate a constant infusion) caused pocket CO_2 to rise and O_2 to fall as expected in vasoconstriction; in 2 hours CO_2 reached 100 mm Hg and O_2 was down to 1 or 2 mm Hg. In contrast, 7 rats given repeated injections of lactic acid, 1 ml every 30 minutes, showed a rise in both CO_2 and O_2; in 1 or 2 hours, CO_2 sometimes went above 100 mm Hg and O_2 increased to slightly above control average. Carbon dioxide changes rapidly in these pockets but the tissue's lower permeability to O_2 causes changes of pO_2 in a 15-20 ml pocket to lag behind an altered tissue tension by 4 to 6 hours(1). Pockets were purposely decreased to 5-10 ml for the epinephrine and acid experiments, but probably a significant O_2 lag occurred nevertheless.

Pockets having had hesperidin, trypan blue, or India ink injections the previous day often contained fluid, but saline-injected pockets (1 ml/day) did not. Untreated subcutaneous gas pockets develop an organized layer of connective tissue and blood vessels within the first week(3); hesperidin treatment caused marked changes of this wall. Macroscopically it assumed a rubbery gelatinous consistency. Histological examinations revealed a number of macrophages loaded with brownish granules in the inner layers of the wall. The granules were strongly PAS positive (as is hesperidin) and gave a negative reaction for iron. Animals treated with trypan blue or India ink had numerous macrophages loaded with these materials scattered throughout the wall. In hesperidin treatment, but not with the other particulates, there was a tendency for macrophages to occur in a well defined layer. When both hesperidin and India ink were injected together, a given macrophage appeared to carry either the PAS positive material or India ink, but not both.

Discussion. The gas tensions after injections of hesperidin, India ink, and trypan blue into the pockets cannot be due to simple alteration of local vasomotor tone or local metabolic rate because O_2 and CO_2 tensions did not change reciprocally.

There are several possible explanations: (a) Energy for phagocytosis is usually ob-

tained from glycolysis, even in the presence of O_2(4). The granules of hesperidin-like material seen in cells of the pocket wall suggest that phagocytosis occurred in the hesperidin-treated pockets, as well as in pockets treated with India ink and trypan blue. The glycolytic process would increase local CO_2 tension directly by CO_2 production without consumption of O_2. Moreover, glycolytic production of acid would tend to increase both O_2 and CO_2 indirectly, CO_2 by liberation from bicarbonates in tissue and blood, and O_2 by liberation from hemoglobin in nearby blood. Production of acid was simulated by introduction of lactic acid into the pocket; increased CO_2 and O_2 resulted.

It is not clear how the injected hesperidin, dissolved in saline, became converted to the apparently insoluble intracellular granules. Possibly the substance was altered by local detoxication or other chemical change.

(b) One of the several actions of hesperidin is to protect epinephrine from oxidation(5). Local excess of epinephrine in the pocket leads to vasoconstriction of nearby capillaries, as was demonstrated by the epinephrine administrations. Because of the lag of O_2, one could conceivably obtain the results in Fig. 1 by sampling during the unsteady state if hesperidin causes a period of vasoconstriction which is followed or preceded by a period of vasodilation. Variability was so great that no conclusive evidence on this point could be adduced from analysis of the data of Fig. 1 by noting pO_2 as a function of time after last hesperidin injection, or from a separate series in which samples were taken every few hours during a series of injections. This epinephrine hypothesis does not help in explanation of the trypan blue and India ink results.

(c) The unusual pocket tensions are compatible with a low lung ventilation combined with high local blood flow. This explanation was discounted by failure to find pCO_2 elevation in subcutaneous gas of 2 rats which received hesperidin intraperitoneally.

(d) Hesperidin appears to modify the permeability of tissues to solutions and particulate material(6,7). In the steady state, O_2 and CO_2 in these pockets are nearly equilibrated with local tissue tensions(1), so it is difficult to see how a permeability change alone could alter the levels to the extent observed.

(e) Injection of acid directly into gas pockets can cause a high CO_2 and normal or high O_2 as shown by the lactic acid experiments. Although none of the other test substances were acidic, it is possible that acid might be liberated from the phosphorylated hesperidin by some reaction in the tissue.

We feel that the phagocytosis hypothesis above is the best explanation of our results. However, we cannot definitely rule out other possibilities. The results, especially with hesperidin, may be due to several factors acting at the same time.

Summary. Injection of phosporylated hesperidin into subcutaneous gas pockets in rats caused high pocket pCO_2 accompanied by normal or elevated pO_2. Similar results from intrapocket administration of particulates, India ink and trypan blue, and of lactic acid suggested that the pocket gas tensions reflected a glycolytic metabolism associated with phagocytosis.

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