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The Use of Radioactive Microspheres to Quantitate Hyperemia in Dermal Inflammatory Sites[1] (39096)

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The four cardinal signs of inflammation are redness, heat, swelling, and pain. Esperimental studies on the nature of the inflammatory reaction, its mediation, and its control by pharmacological agents, have used various assays and parameters to measure the inflammatory response. The hyperemia has been assessed in qualitative terms, or, in species such as the guinea pig, by measuring the diameter of the skin erythema. It is clear that objective and quantitative measurements of hyperemia are a necessary prerequisite to the study of the involvement of known or suspected mediators that act on the microcirculation.

The measurement of blood flow, even to large organs, has been a technical problem without simple solutions. The introduction of the use of radionuclide-labelled, carbonized microspheres by Rudolph and Heymann (1) has overcome much of the difficulty, and subsequently, this method has been investigated in sufficient detail to conclude that it is a reliable method of measuring blood flow to the large and small organs of the dog, sheep, and rabbit, providing certain conditions are met (2–5). These more comprehensive studies should be consulted for details concerning the advantages and limitations of the use of microspheres.

These spheres are of a diameter such that they lodge in the microcirculation following their introduction into the arterial, blood vascular system. With appropriate choice of size, microspheres have been shown to have negligible recirculating capacity; and, with appropriate doses, they have little effect on the gross physiological parameters of the animal.

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The present studies have been aimed at determining the feasibility of using this method to measure the flow of blood to localized dermal lesions in the rabbit, guinea pig, and sheep. It was the aim to measure not only comparative differences between agents or the dose injected, but also to assess the possibility of measuring the blood flow in absolute units, namely, ml/min/gram.

Materials and methods. Randomly bred, female guinea pigs (600–800 g) and rabbits (2–3 kg) were anesthetized with urethane (BDH). Lidocaine hydrochloride 1.0% (Xylocaine, Astra) was used as local anesthetic when required. A P.E. 100 polyethylene catheter drawn out to approximately one-half of its original diameter was advanced 6 cm into the right carotid artery. The tip of the catheter was advanced to the ascending aorta and sutured in place with silk. A 19 gauge needle was used to introduce the microspheres into the large end of the catheter. For experiments on sheep (6–8 months), randomly bred ewes were injected with sodium pentabarbital, and Red Kifa catheters were fluoroscopically positioned just above the aortic valve. Microspheres labelled with ^{85}Sr, (10 mCi/gram) of 15 μm $\pm$ 5 μm diameter, suspended in 10% dextran (3M Nuclear Products Division, London, Ont.), were introduced through these catheters. In rabbits approximately 3×10^6 spheres were injected in a volume of 0.6 ml (0.4 ml microspheres plus 0.2 ml 10% dextran). Sheep received approximately double this dose and guinea pigs approximately three-quarters of the rabbit dose. Animals were sacrificed with Nembutal introduced through the catheter and, following the removal of the skin of the back, a 15 mm diameter cork borer and a mallet were used to punch out the injection sites. The skin discs were placed in plastic tubes and the

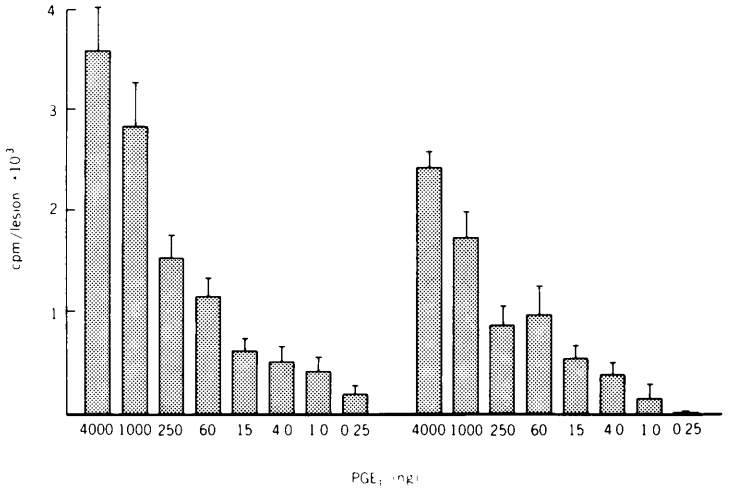

FIG. 1. Localization of ^{85}Sr microspheres in skin sites following intradermal injection of PGE_1. Volumes of 0.2 ml in serial, fourfold dilutions ranging from 4000 to 0.25 ng/site were used. Columns on the left represent the arithmetic mean ± SE of 17 trials in eight rabbits. Columns on the right represent the arithmetic mean ± SE of three trials in one rabbit.

radioactivity counted in a Nuclear Chicago gamma spectrometer. Approximately 12 animals of each of the three species have been examined.

Results. Hyperemia induced by 0.2 ml intradermal injections of various doses of histamine, bradykinin and prostaglandin E_1 (PGE_1) was investigated in rabbits by injecting the microspheres approximately 15 min after the intradermal injections. PGE_1 appears to be the most potent mediator of hyperemia. Fig. 1 shows the relationship between the amount of PGE_1 injected and the number of spheres (cpm) trapped. Values obtained in a typical trial using only one animal are compared with the mean values from several trials in groups of animals and an estimate of the errors and reproducibility of the method can be deduced. When comparing more than one animal, mean values obtained from saline control sites were subtracted from all other values. Whereas the injections of the mediators and the microspheres were carried out using anesthetic, in small animal experiments it is also feasible to use unanesthetized animals in which catheters have been placed previously.

Figure 2 shows the random variation in different regions of the back of a rabbit. In the area extending from the scapulae to the iliac crests, and bounded by mid-axillary lines laterally, four squares were drawn. A constant dose (1 μg) of PGE_1 was injected in nine sites in each of the squares. Providing the catheter was correctly positioned, the variation obtained in any species so far examined was random. Furthermore, the symmetrical distribution of microspheres to the left and right kidney indicated that adequate mixing with the blood had occurred. In seven rabbits the difference between the

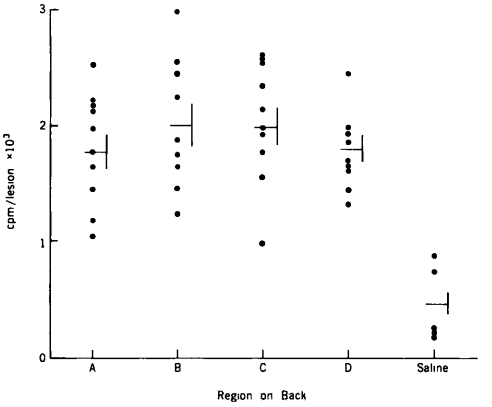

FIG. 2. Distribution of ^{85}Sr microspheres in 36 sites following injection of 1 μg PGE_1 per site into a single rabbit. The skin of the back was divided into the quadrants A, B, C, and D. Saline sites were randomly distributed. The individual values mean and SE is given.

left and right kidneys was 4.49 ± 0.84% of the total count in both kidneys.

Delayed hypersensitivity reactions have been studied in the guinea pig traditionally, due to the characteristic erythematous lesion that develops. Fig. 3 compares the mean number of radioactive counts obtained in the skin of sensitized guinea pigs that had been injected with serial dilutions of purified protein derivative (PPD). The number of microspheres trapped was dependent on the dose of PPD injected and increased in relation to the degree of erythema. The maximum diameter of the erythema reached was 12 mm. Severe lesions tended to penetrate deeper into the dermis, and assessment solely in terms of diameter of the erythema was unsatisfactory; however, the measurement with the microspheres took this into account.

In sheep it has been possible to quantitate the blood flow to single lymph nodes as well as to a variety of dermal lesions (Hay and Hobbs, to be published). In Table I the number of spheres which were trapped in

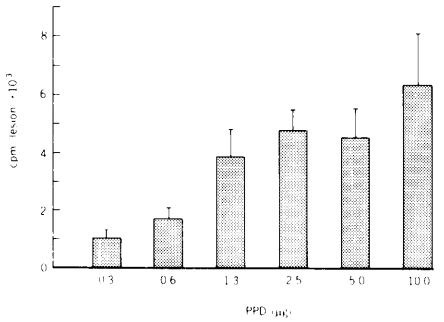

FIG. 3. Localization of ^{85}Sr microspheres in tuberculin reactions in the skin of guinea pigs. The mean ± SE of eight sites in four animals is represented. Animals were sensitized with BCG 1 month before skin testing with various doses of PPD. Microspheres were injected 24 hr after the PPD.

5-day-old cellular hypersensitivity lesions is compared with normal skin. When the total number of injected spheres is known, these values can be expressed as a percentage of the cardiac output (4). For example, assuming a cardiac output of 6 liters/min in this animal, the flow to the lesions was

$$22.4 \times \frac{6883 \text{ cpm/g}}{10^6 \text{ cpm (total injected)}} \times 6 \text{ liters/min} = 1.84 \text{ ml/min/g}.$$

Discussion. When radiolabelled microspheres were introduced into the arterial system of animals with dermal erythematous lesions, a proportion of the microspheres were trapped in the lesions. The number of microspheres trapped increased in proportion to the severity of lesions produced by increasing doses of either PGE_1 or tuberculin. Since the hyperemia observed in these experiments blanched when the animals were sacrificed, it was concluded that the trapping of microspheres indicated true hyperemia and not hemorrhage. In rabbits it was feasible to inject approximately 40 individual sites in the back of the each animal to test antigens, mediators, or pharmacological products for their capacity to affect local blood flow. When unknown substances were injected in triplicate, a standard error of less than 20% of the mean radioactivity measured could be obtained. Several factors should be considered when comparing the results obtained from individual animals or when pooling results from more than one animal. Corrections should be made for isotope decay and variations in the specific activity of different shipments of microspheres. It has been found that the number of microspheres that localized in the skin did not always correlate with the number that

TABLE I. LOCALIZATION OF MICROSPHERES IN SHEEP TISSUES[a]

Normal skin (4),[b] cpm/g	NLT reaction skin (4), cpm/g	Normal lymph nodes (6), cpm/g	Left kidney (76.7 g), cpm/g	right kidney (69.7 g), cpm/g	Spleen (59.8 g), cpm/g
280 ± 19	6883 ± 691	9246 ± 2129	19,374	19,327	5334

[a] A comparison of the distribution of localized ^{85}Sr microspheres in various tissues of a sheep. Normal lymphocyte transfer (NLT) lesions were produced by the intradermal injection of 2×10^7 allogeneic lymphocytes 5 days before injecting the microspheres.

[b] Number of test sites or number of nodes.

localized in the kidneys and spleen, presumably due to differences in skin temperature and autonomic control. Therefore, it is preferable to take into account the number of microspheres trapped in the area of normal skin surrounding the punched out lesions. Since this may involve up to 40 g of skin, a large proportion of radioactivity is measured, thereby improving the sampling and counting errors.

With experience in the positioning of the catheter in the small animals, a satisfactory distribution of microspheres was realized in approximately 80% of the animals. If the catheter end is not advanced far enough into the ascending aorta, a disproportionate number of microspheres will be trapped in the head and thorax. Routinely, both kidneys, spleen, and sometimes eyes were removed and counted to check the distribution. Random positioning of injection sites in the back provided a second check on the uniformity of distribution, since it enabled a comparison of the similarity between saline injection sites.

These experiments have demonstrated that prostaglandins of the E type are potent mediators of hyperemia. Significant hyperemia was produced with 1 ng of PGE_1 ($P < 0.05$ using an independent t test to compare the difference between mean values for prostaglandin and saline sites). On an equal weight basis, PGE_1 was considerably more potent than either histamine or bardykinin. By administering ^{125}I-labeled albumin concurrently with ^{85}Sr-labeled microspheres, the effects of prostaglandins on the enhancement of vascular permeability by classical mediators has been examined (Johnston and Movat, to be published).

Cellular hypersensitivity lesions have a rich blood supply as shown by the number of microspheres trapped in tuberculin reactions and normal lymphocyte transfer reactions. In other experiments it has been possible to trap approximately 7000 microspheres in single lesions in the guinea pig by injecting PGE_1 directly into previously established tuberculin reactions. The capacity for a small region of skin to increase its blood flow is therefore very large.

Studies in progress using this technique are aimed at defining the role of hyperemia in inflammation, hypersensitivity, and the immune response.

Summary. A variety of dermal lesions produced by PGE_1, tuberculin, and allogeneic lymphocytes were induced in rabbits, guinea pigs, and sheep, respectively. Microspheres labeled with ^{85}Sr were injected into the ascending aorta, and the distribution of radioactivity in the lesions, saline control sites, and various major organs was assessed. It was concluded that the trapping of the microspheres was related to the degree of hyperemia induced, and that this technique enabled a quantitative measure of the blood flow. PGE_1 was found to be a potent mediator of hyperemia and to have a significant effect on the blood flow at a dose of 1 ng. The blood flow to cellular hypersensitivity reactions was measured and the capacity of the skin to accomodate a large increase in flow during the inflammatory response was established.

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