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Studies on Regulation of Plasma Iron in Dogs with Turpentine Abscesses. (27493)

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Hypoferremia occurs acutely in both man and animals as a result of sterile or pyogenic inflammation(1,2). The drop in plasma iron occurs within the first 24 hours after onset of inflammation and persists for the duration of inflammation. It has been shown that radioactive iron administered to animals with acute inflammation in amounts exceeding the iron binding capacity of the circulating plasma is taken up largely by the reticuloendothelial systems of the liver and spleen (3) as is the iron of senescent red cells(4). The studies by Freireich(4) indicate that during acute inflammation there is a defect in the release of iron from the tissues to the plasma. The mechanisms responsible for these changes in iron metabolism and for the resulting hypoferremia are unknown. This phenomenon—a local inflammatory process acting at a distance upon target tissues—suggested that a humoral factor might mediate the alterations in iron distribution responsible for this hypoferremia.

Preliminary investigations by Cartwright *et al.*(1) suggested that the sterile empyema fluid produced in a dog by intrapleural installation of turpentine induced a lowering of the plasma iron when injected into normal dogs. This report and the above considerations prompted a search for a potent circulating factor in dogs with subcutaneous turpentine abscesses which would be capable of lowering the plasma iron of normal recipient dogs when administered in relatively small volumes.

Materials and methods. Sterile abscesses were produced in mongrel dogs (weighing approximately 11 kg) by subcutaneous injection of 2 ml of turpentine at the base of the scapula. Blood was collected in acid citrate-dextrose solution from normal dogs and from dogs with sterile abscesses, and plasma was separated by centrifugation at 3000 rpm at room temperature. If not used at once, plasma was frozen and stored at −20°C.

The animals were in the fasting state at time of injection of turpentine, collection of blood and infusion of plasma. Bacterial cultures of plasma used for infusion were negative. Recipient animals did not have post infusion pyrexia or other ill effects. Plasma iron levels were determined by the method of Schade *et al.*(5).

Results. Fasting morning serum samples were obtained from 3 normal dogs over a 15-day period and iron levels were determined. The results are shown in Fig. 1. A marked fluctuation in iron level was noted; in one case as much as 200 μg per 100 ml from one day to the next.

In another series of experiments, 45 ml of acute phase plasma (approximately 8% of re-

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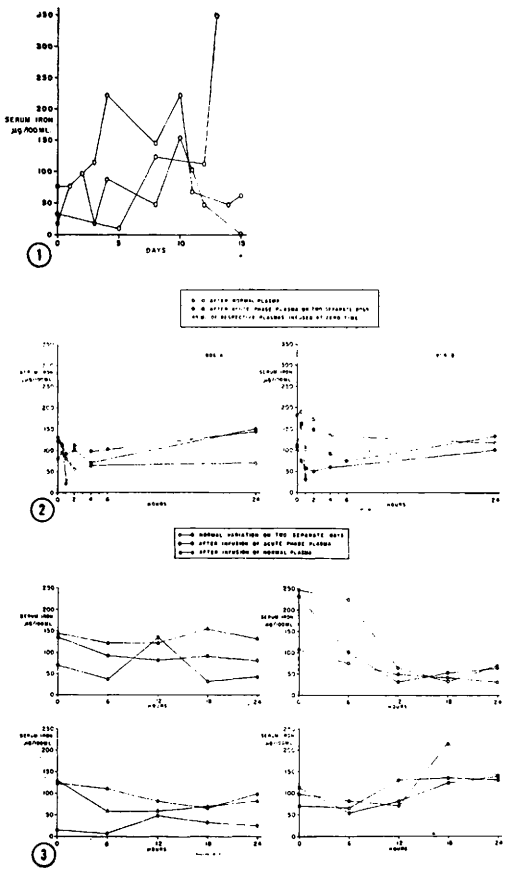

FIG. 1. Serum iron fluctuations in normal dogs.
FIG. 2. Infusions of normal and acute phase plasmas from one donor dog into 2 recipient dogs.
FIG. 3. Multiple infusions of normal and acute phase plasmas from 2 donor dogs into 4 recipient dogs. Total of 200 ml of respective plasmas infused in 4 separate infusions at zero, 6, 12 and 18 hours.

cipient's plasma volume) obtained 24 hours after induction of a turpentine abscess at the time of maximal hypoferremia was injected on each of 2 different days into a normal dog. As a control the recipient dog, on another occasion, received the same volume of normal plasma, obtained from the same donor dog at a time when he had no abscesses. Fig. 2 shows the findings in 2 such dogs. Marked variation in plasma iron level was noted following the infusion of normal plasma and no significant difference from these variations followed infusion of acute phase plasma. The same results were obtained when dogs served as *both* donors and recipients of their own normal or acute phase plasma. No significant alterations in plasma iron levels were found in rabbits infused with dog acute phase as compared to plasma iron levels after infusions of normal plasma. Similar results were obtained when normal Rhesus monkeys were infused with acute phase and normal plasma obtained from other monkeys.

Fig. 3 depicts the response of normal recipient dogs to *repeated* infusions of 50 ml aliquots of normal or acute phase plasma (approximately 36% of recipient's plasma volume) from other donor dogs administered four times in an 18-hour period. No meaningful depression of the plasma iron level occurred compared to the normal variation observed.

Discussion. Sustained hypoferremia occurs in mongrel dogs given turpentine abscesses. However, in healthy dogs there may be marked daily and hourly fluctuations in plasma iron levels. Therefore, data suggesting a temporary hypoferremic effect induced by infusion of a body fluid from a dog with a turpentine abscess into a normal dog must be viewed with suspicion. In this study rabbits and monkeys were also used as recipients in order to circumvent this variability since these animals were found to have a more constant daily morning plasma iron level.† Meaningful hypoferremic effects were not obtained in these animals. In an attempt to overwhelm the normal fluctuations of plasma iron, multiple infusions of acute phase plasma were given to dogs, but even this stimulus failed to reveal a circulating factor capable of lowering the plasma iron level.

Conclusions. Marked hour to hour and day to day fluctuations in plasma iron levels were observed in normal mongrel dogs. It was not possible to demonstrate a factor in the plasma of dogs with subcutaneous turpentine abscesses that was capable of producing hypoferremia in normal recipient dogs.

† The iron method itself might be suspect in the dog experiments. However, the stability of iron levels in rabbits and monkeys as well as a satisfactory correlation seen between iron levels and patients with anemia of iron deficiency and chronic infection exclude this.

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Effects of Natural Estrogens on L Strain Fibroblasts in Tissue Culture.* (27494)

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Several hormonally active steroids inhibit growth of tumor cells(1), microorganisms(2) and tissue cultured cells(3). The most frequently observed physiological effects on microorganisms are involved with suppression of cell growth, or cell function such as ion, sugar or amino acid accumulation(4). Holden and Adams have described the inhibitory reaction by corticoids on L strain fibroblasts (5), and estrogens have been shown to exert an inhibitory action on HeLa cells comparable to that induced by corticoids(6). The L strain system, because of its accessibility to quantification in a serum free medium, was chosen as a model animal cell to study *in vitro* to clarify the nature of the primary interaction between mammalian cells and estrogens.

Materials and methods. A replicate series of L strain (929) fibroblast cultures was set up in 150-ml milk dilution bottles; a series consisted of approximately 100 bottles. Each culture was established by inoculating a 10-ml portion from a large suspension containing about 3.0×10^5 cells/ml in medium 199 supplemented with 0.5% bacto-peptone (199P) (7). After 24 hours at 37°C, the populations of cells attached to the glass reach the early exponential phase of the growth cycle; at this time the used medium was decanted, and 10 ml of fresh 199P medium containing the test steroid was placed on the cell sheets.

Estrone, estradiol-17β and estriol were dissolved in propylene glycol at a concentration of 1 mg/ml. These stock concentrates were sterilized by autoclaving. Chromatography in a 2 phase system showed that the steroids were not altered(8). A 1:100 dilution of the stock in medium 199P gives an estrogen concentration of 10 μg/ml; this is 3.5×10^{-5} M for each of the estrogens. The limiting solubility for estrone and estradiol in this medium is about 20 μg/ml; estriol is more readily soluble. Dialyzed horse serum was added to medium 199P in some experiments which increased the solubility of these steroids, and at a 20% serum concentration, the solubility of estrone and estradiol was doubled. Experiments were controlled by setting up a series of cultures which were bathed in 199P containing 1% propylene glycol.

At the time the medium containing the test steroids was added to a culture series, 9 bottles were set aside, and harvested as the zero time controls. At subsequent 24-hour intervals, through a period of 6 days, 9 bottles from the control set and 9 bottles from the test set were harvested. In harvesting, the medium was decanted from the bottles and centrifuged to pack the cells that became detached from the glass at the higher estrogen concentrations. The bottles were allowed to drain onto a paper towel for a few minutes. Three milliliters of cold 5% TCA was added to each to bathe the cell sheet. The cells were then scraped from the glass into the cold TCA and this precipitated suspension was combined with its respective pack of detached cells. The contents of 3 bottles from each set were combined in a 15-ml conical centrifuge

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