Table 3. Summary of observations on the discharge of immature polymorphonuclear leukocytes (1-lobe form) following injections of LPF after repeated administration of compound F into inflamed area. The figures are averages of five observations in each control and experimental group.

	Control dog	Experimental dog
Total amount of saline or of compound F administered	5.9 ml	19.4 mg
Basal number of 1-lobe form	18.4%	22%
Highest number of 1-lobe form following injection of 15 to 20 ml of LPF	54.4%	33.6%

interacting directly with the preformed LPF at the site of inflammation.

In the case of leukotaxine, the sole observations reported here do not justify a similar inference, inasmuch as earlier studies had demonstrated that leukotaxine *per se* mixed *in vitro* with cortisone or compound F is to a large extent inactivated (*2, 12*). It is, of course, conceivable that both direct interaction with leukotaxine by the corticosteroid and suppression of formation of this substance in injured cells may occur. It is scarcely conceivable that any of the difficultly soluble corticosteroid would be carried along in the chemical extraction of leukotaxine from the exudate (*6, 12*). This likewise would hold for the extraction of the LPF where exclusively a protein "salting out" process with $(NH_4)_2SO_4$ is utilized (*7*). Furthermore, the *in vitro* observations with the LPF described here indicate that the corticosteroid has no effect on the LPF *per se*.

In conclusion, the evidence briefly reported here indicates that the anti-inflammatory mechanism of compound F can be explained as acting at a cellular level (in distinction to molecular, enzymatic, or other levels). The activity of the injured cell is impaired, so that it becomes less effective in producing some, if not all, of the specific chemical factors involved in inflammation.

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Occurrence of a Pungent Insecticidal Principle in American Coneflower Roots

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A number of insecticidal unsaturated isobutylamides have recently been isolated from natural sources (*1*). They all possess a high order of pungency. A small amount placed on the tongue causes profuse salivation and a burning, paralyzing effect on the tongue and mucous membranes. They are very closely interrelated structurally, the acid components containing an unbranched chain of 10 to 18 carbon atoms and at least two double bonds.

During an investigation of scabrin (*2*), one of the most active of these compounds, which occurs in the roots of *Heliopsis helianthoides* var. *scabra* (family Compositae), it was learned (*3*) that the roots of the American coneflower, *Echinacea angustifolia* DC., a closely related plant, are highly pungent when chewed. A search of the literature revealed that this property had previously been noted (*4*) and that an acetone extract of the roots contains a mosquito larvicide (*5*). In addition echinacoside, $C_{35}H_{46}O_{20}$, which was isolated from the methanol extract of the roots by Stoll *et al.* (*6*), possesses antibacterial properties. The American coneflower is indigenous to Kansas, Nebraska, and Missouri. The roots are used medicinally in the healing of wounds and inflammations and are available commercially.

The dried roots (*7*) were ground and extracted successively in a Soxhlet extractor with *n*-pentane, ethyl ether, chloroform, and ethanol, and the solvent-free extractives were tested separately in refined kerosene solution against adult houseflies, *Musca domestica* L., by Norman Mitlin. Only the pentane extractive, consisting of 4 percent of a highly pungent yellow oil, was toxic to these insects, showing high knockdown and fair mortality. A considerable quantity of an inactive colorless, liquid hydrocarbon, bp 85°C (0.5 mm-Hg), N_D^{25} 1.4488, was obtained from the extractive by distillation. These constants correspond to those reported by Woods (*8*) for the unidentified hydrocarbon $C_{15}H_{28}$ isolated previously from *E. angustifolia* roots. The activity was found to be concentrated in the neutral fraction, a viscous yellow oil that solidified when cooled to 5°C, after separation of the inactive mixed-acids fraction from the distillation residue. By dissolving the solid in petroleum ether (bp 60° to 80°C), cooling the solution at −78°C, filtering off the resulting amorphous solid, and recrystallizing it several times from the same solvent at −10°C, there was obtained 250 mg (0.0004 percent of the dry root) of colorless needles, mp 63° to 64°C. This material had the characteristic numbing effect on the tongue and possessed moderate insecticidal activity in tests with houseflies. The compound has been designated *echinacein*.

Attempts to identify echinacein were greatly hampered by the fact that the crystals are highly unstable, polymerizing in the air after 1 hr at room temperature and after 2 days in a nitrogen atmosphere at −10°C (a natural antioxidant is apparently present in the crude extract of the roots). The compound showed a maximum in the ultraviolet at 259 mμ, characteristic of a conjugated triethenoid structure, and analyzed fairly well for $C_{16}H_{25}NO$. Hydrogenation in ethanol solution with a platinum catalyst gave colorless needles, mp 51° to 52°C, identical with N-isobutyllauramide. Permanganate oxidation of echinacein resulted in a mixture of acids, none of which were present in sufficient amount for identification. One of these acids, volatile with steam, consisted of a few drops of a colorless, highly corrosive oil with a penetrating unpleasant odor. Accidental contact of a trace of this acid with the skin of the hand caused an immediate burning sensation and rapid blistering of the skin at the site of contact, followed by peeling of the skin after 2 days.

Echinacein appears to be the isobutylamide of a highly unsaturated 12-carbon straight-chain acid and may be identical with neoherculin, mp 63° to 65°C, isolated by Crombie (*9*) from the bark of *Zanthoxylum clava-herculis*. Further identification must await the isolation of larger quantities of pure material.

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Utilization and Intestinal Excretion of Calcium in Man

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Data on Ca^{45} metabolism in man have been reported and measurements of endogenous fecal calcium and the utilization of ingested calcium have been discussed (*1*). In this study the rate of absorption and utilization of ingested calcium, the excretion of digestive juice calcium into the gastrointestinal tract and its reabsorption were measured with the Ca^{45} technique and metabolic balances (*2*). Two patients without bone disease, B and L, maintained on low calcium diet, received a single dose of 50 μc Ca^{45} with 30 mg of calcium carrier orally. Serum, urine, and stool were analyzed for calcium and Ca^{45} (Fig. 1). Balances of calcium, phosphorus, and nitrogen were measured for four 6-day periods (Table 1).

The top graph of Fig. 1 shows the specific activities of serum and urine and the serum Ca^{45} levels of patient B in the first 24 hr. Radioactivity was first detected in blood at 15 min; a 5-min sample had no activity. The specific activities of blood and urine from the second day to the termination of the experiment are plotted on a semilogarithmic scale in Fig. 1. There is a simple exponential decline with a half-time of 8 days. The first stool collected at 2 hr contained no activity; the next specimen at 25 hr, 5.0 percent; after a 5-day period a total of 58 percent of the dose was excreted. The specific activity of these samples is not plotted, since it was considerably higher than 1 percent of the dose per 100 mg of Ca. Figure 1 shows

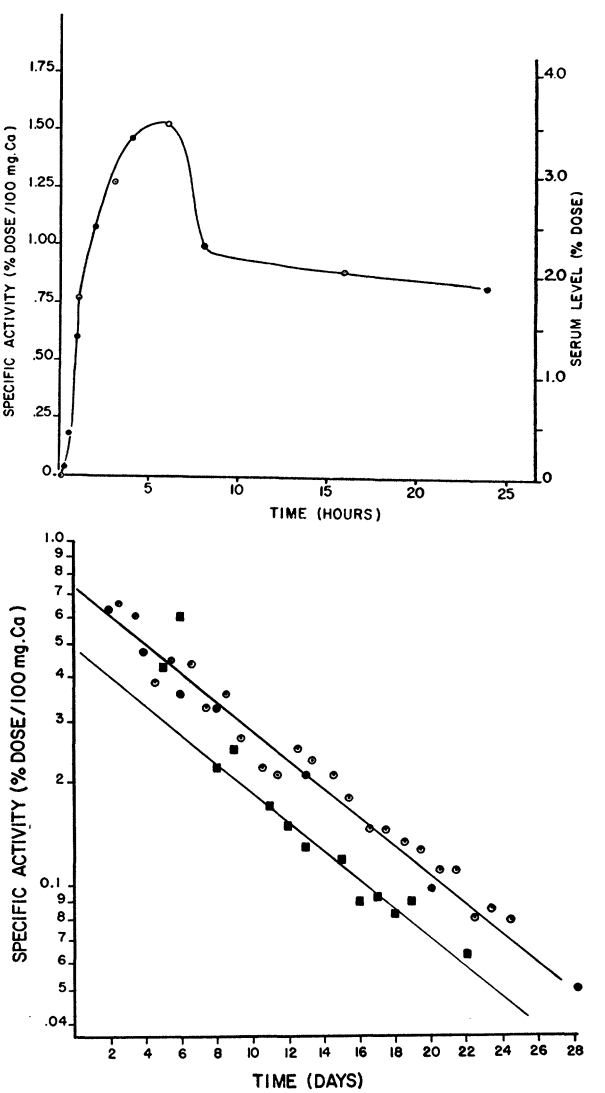

Fig. 1. Calcium and Ca^{45} content in serum, urine, and stool of two human patients. Symbols: ● plasma, ⊙ urine, ■ stool.