R. rubrum with N_2 as the sole or primary nitrogen source are still unknown, comparable experiments with such cells have not yet been possible. These nutritional requirements are now under investigation.

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On the Anti-inflammatory Mechanism of Hydrocortisone (Compound F)

Valy Menkin

Agnes Barr Chase Foundation for Cancer Research, Temple University School of Medicine, Philadelphia

My earlier studies (1940, 1942) have indicated that adrenocortical extract or cortisone suppresses the increased permeability of small blood vessels caused by an alkaline exudate or its contained leukotaxine. Subsequently, it was shown that cellular activity, as gaged by the incidence of cell division, was considerably reduced in the eggs of the sea urchin, *Arbacia punctulata* by the presence in sea water of corticosteroids (*1–6*). Severely injured cells, such as those encountered in acute inflammation, liberate, as a result of their activity, numerous chemical factors capable of reasonably explaining the various manifestations of inflammation (*7*). These factors include, among others, leukotaxine and the leukocytosis-promoting factor (LPF). Leukotaxine explains the initial increased small blood vessel permeability and the migration of polymorphonuclear leukocytes into an inflamed area (*7, 8*). The LPF induces a discharge of leukocytes from the bone marrow and in part explains the mechanism of leukocytosis often accompanying an acute inflammation (*7*). Observations were undertaken (*9*) to determine whether the presence of compound F in an inflamed area would suppress the activity of injured cells, so that they no longer would be able to produce adequate amounts of active leukotaxine or of the LPF (*10*).

Acute inflammation was induced in dogs by the intrapleural injection under pentobarbital anesthesia of 1.5 ml of turpentine. The experimental animals were then injected at the same site with a suspension in saline of 10 to 20 mg hydrocortisone (compound F, free alcohol) (*11*), repeated at daily intervals for 3 to 4 days. The control dogs, following the intrapleural injection of turpentine, received daily injections of saline into the inflamed area. The increased permeability of the small blood vessels was determined, as was previously described, by the extent of accumulation of intravenously injected trypan blue into the treated cutaneous areas on the abdomen of rabbits (*1–4, 6*).

Leukotaxine was extracted, as is described elsewhere, from a given sample of exudate withdrawn from the chest cavity of experimental and control dogs (*12, 13*). Following repeated injections of hydrocortisone (compound F) into the inflamed area, the activity of leukotaxine in regard to its capacity of increasing small vessel permeability was considerably reduced (Table 1, Fig. 1). The outward migration of leukocytes

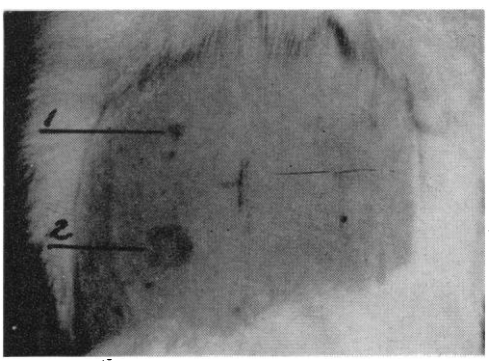

Fig. 1. Inability of leukotaxine derived from compound F-treated exudate to induce increased permeability of small blood vessels. Rabbit 21–19: *Area 1*, 0.4 ml of leukotaxine extracted from exudate of dog 254-T following two successive daily injections of compound F into inflamed area. Total of about 42 mg of hydrocortisone in 6 ml of saline injected into inflamed pleural cavity of the dog from which leukotaxine had been extracted from a given quantity of exudative material. *Area 2*, 0.4 ml of leukotaxine extracted from exudate of dog 255-T following two successive injections of a total of 6 ml of saline into inflamed area. Leukotaxine extracted from an identical quantity of exudative material as in the case of dog 254-T. At end of experiment 6 ml of 1-percent trypan blue in saline was injected intravenously. The injections of areas 1 and 2 were made in the dermis of the abdomen.

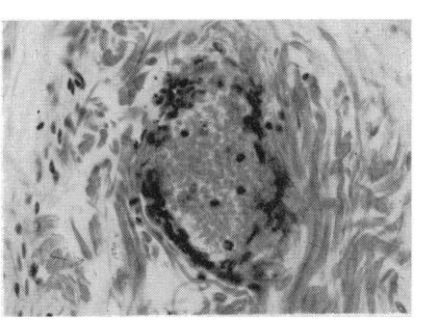

Fig. 2. Effect of leukotaxine in inducing margination of polymorphonuclear leukocytes in skin of rabbit 21–22. Taken about 1 hr after intracutaneous injection of leukotaxine extracted in turn from a sample of canine exudate. The exudate was derived from the pleural cavity of a dog previously injected with turpentine into that region. The animal had received two successive daily injections of saline, each 3 ml. The section is that of a small blood vessel, possibly a venule. (×200)

Table 1. Inhibition of increased capillary permeability caused by leukotaxine upon repeated injections of compound F into an inflamed area. Commercial hydrocortisone was used with rabbits 21–21 and 21–22; compound F (free alcohol) was used in all other experiments.

Rabbit	Compound F injection		Total vol. of saline inj. into inflamed area of control dog (ml)	Accumulation of trypan blue in cutaneous areas treated with	
	No. of inj. into original area of inflammation	Total amt. inj. into inflamed area (mg)		Leukotaxine from compd. F-treated exudate	Leukotaxine from control saline-treated exudate
21–27	1	24	4	3+	2+ to 3+
21–18	1	21.9	3	Trace	+
21–31	1	11		2+	
	3	37.6	8	Trace	2+
21–19	2	42	6	0	3+
21–21	2	40	6	0	2+ to 3+
	2	40	6	Trace to +	
21–22	2	40	6	Trace	+

Table 2. Summary of observations on the effect of the LPF of exudates following repeated injections of compound F (free alcohol) into an inflamed area. Commercial hydrocortisone was used with dogs 249-T and 264-T; compound F was used in all other experiments.

Dog	Control dog		Experimental dog		
	Amt. of LPF inj. (ml)	Rise in white blood cell count (%)	Total amt. of compd. F used (mg)	Amt. of LPF inj. (ml)	Rise in white blood cell count (%)
256-T	30	158.1	41.9	30	89.0
8-D	±18	77.6	20.0	20	51.4
171-T	20	62.2	20.0	20	30.6
267-T	±23	78.2	24.0	25	31.8
282-T	±23	88.1	11.0	24	34.3
249-T			40.0	21	55.7
264-T			40.0	20	48.0
χ			34.2	30	50.3
Avg.		92.8			48.9

as induced by leukotaxine is also conspicuously diminished following repeated injections of compound F into the inflamed area (Figs. 2 and 3). Diapedesis of leukocytes occurs through both capillaries and venules; and this, in contrast to the findings in controls (Fig. 2), is relatively absent with the use of leukotaxine extracted from experimental exudates (Fig. 3).

The LPF was extracted, as is described elsewhere (*7*), from a given sample of alkaline exudative material obtained from control and experimental dogs. The results are summarized in Table 2. It is clear that the

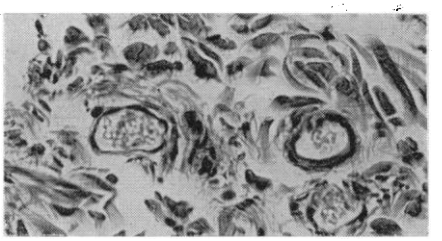

Fig. 3. Small blood vessels in skin of rabbit injected with leukotaxine extracted from an exudate of a dog that had previously received two successive injections of compound F into its inflamed pleural cavity. The small blood vessels appear to be a capillary on the left and possibly an arteriole on the right. Diapedesis of leukocytes through the capillary wall is absent. (×200 approx.)

repeated local injections of hydrocortisone (compound F) into the inflamed area yielded in the exudate an LPF, on the average about half as potent as that obtained from control samples. The course of one such experiment is illustrated in Fig. 4. The discharge of immature polymorphonuclear leukocytes (1-lobe form) is likewise inhibited by compound F administration (Table 3).

The LPF when mixed *in vitro* with compound F is not altered in its capacity to increase the number of circulating leukocytes. This was demonstrated in three separate experiments by adding 15 to 21 mg of compound F to about 50 mg of LPF and maintaining the mixture *in vitro* for 18 to 19 min prior to intravascular injection into dogs. There was no reduction in the activity of the LPF, the experimentally treated dogs inducing an average rise in the WBC of 130.1 percent as against 111.4 percent in the controls. This evidence supports the view that hydrocortisone injected directly into the inflamed area suppresses the activity of the injured cells in forming the LPF rather than

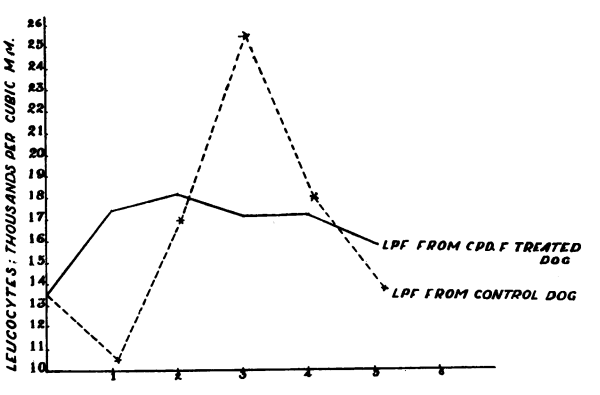

Fig. 4. Partial inactivation of the LPF of exudate by direct injections of compound F into the inflamed area of dog 282-T. The amount of LPF tested in this experiment was recovered from 8 ml of exudate derived either from a compound F-treated dog or from the control sample of exudative material. [Graph prepared by William Rogers]

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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27 August 1954.

Occurrence of a Pungent Insecticidal Principle in American Coneflower Roots

Martin Jacobson

Entomology Research Branch, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland

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*.