muscularly daily) was given to 3 dogs with Heidenhain pouches for a period of 37 days. Maximal acid and pepsin output in response to histamine and gastrin increased during hydrocortisone administration. During control studies, the mean maximal acid output in response to gastrin was lower than to histamine but the maximal responses to these 2 stimulants were generally equal during hydrocortisone administration. In contrast, mean maximal pepsin output was higher with gastrin during control studies and remained higher than histamine during administration of hydrocortisone.

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Effect of Non-Steroidal Anti-Inflammatory Drugs in the Evans Blue Pleural Effusion. (31582)

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The Evans Blue pleural effusion method has been suggested and used as a test for anti-inflammatory compounds. As generally used(1,2,3) drug effect is studied by measuring the volume of pleural fluid 6 hours following the intrapleural injection of the dye. Further investigation of the method indicated that the exudative response obtained later than 12 hours after injection of the irritant was significantly greater than at 6 hours, and that there were certain advantages to using the volume at this time in assaying drug activity. Results obtained with this modification are presented here for acetylsalicylic acid, phenylbutazone, indomethacin, mefenamic and flufenamic acids.

Methods and materials. Male Holtzman rats (250-400 g) were randomized into the control and experimental groups using random tables(4). Compounds were administered by

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intragastric intubation (1 ml/100 g body weight) as a suspension, utilizing a few drops of Aquet,† a synthetic detergent, as a suspending agent. The control group received the vehicle alone. One hour later, 5 ml of .075% Evans Blue solution‡ at 37°C was administered intrapleurally to lightly etherized rats. The injection was made into the lower right side of the animal through a 26 gauge needle with its length reduced to 6 mm by inserting it through a rubber disc. At different times, selected on the basis of convenience in carrying out the experiments from day to day, the animals were sacrificed with chloroform. Pleural fluids were removed by mild suction, collected in a calibrated centrifuge tube and measured. Data from grossly bloody exudates were not included. The investigator removing the exudate was unaware of the treatment each animal had received. The results were expressed as the average percent change in volume of pleural exudate over the control. A significant reduction in the exudative response over the control as determined by the Student t-test indicated anti-inflammatory activity.

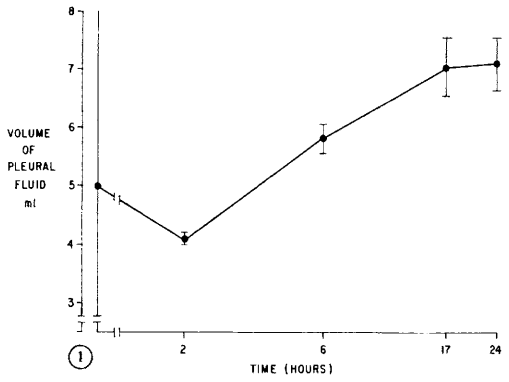
A comparison of the duration of anti-inflammatory activity of flufenamic acid and phenylbutazone was made in a parallel study. Six animals were used in each group, and the volume of pleural exudate was measured at 6, 12 and 17 hours following injection of the dye or 7, 13 and 18 hours following oral administration of the compounds.

Statistical procedures were carried out by the methods described by Bliss(4) and Snedecor(5).

The following compounds were used: phenylbutazone, indomethacin, flufenamic, mefenamic and acetylsalicylic acids.§ The particle size of the acetylsalicylic acid ranged between 7 and 12 μ.

Results. Fig. 1 shows the inflammatory response to the intrapleural injection of Evans Blue. Fluid is initially absorbed followed by an effusive or inflammatory phase attaining peak effect early in the day following injection of the irritant as demonstrated by the fact that the volume of exudate was apparently greatest at 17 hours. The volume of pleural exudate varied from day to day. In 10 control groups (6 or 7 animals per group), the average volume of pleural exudate ± S.E. at 6 hours was 4.2 ± .3 ml ranging from 3.1 to 5.8 ml. At 17 hours, the effusive response was consistently higher. In 69 groups (6 to 8) animals per group), the average volume ± S.E. was 7.9 ± .12 ml ranging from 5.2 to 9.8 ml of pleural exudate. Fifty-six or 87.5% of these were between 6.9 and 9.0 ml.

In view of the consistently higher volume of pleural exudate at 17 hours, anti-inflammatory compounds were evaluated at this in-



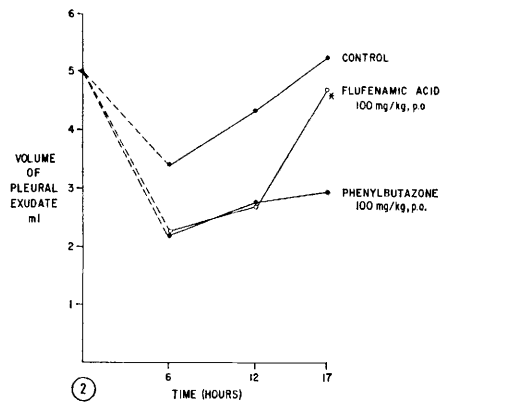


FIG. 1. Pleural response to injection of 5 ml of .075% Evans Blue. Each value is mean ± standard of error of 7 animals. Eight animals were in the 24-hour group.

FIG. 2. Parallel assay comparing anti-inflammatory activity of phenylbutazone with flufenamic acid in the Evans Blue pleural effusion. Each value is mean of 6 animals. Due to a poor injection, there were 5 animals in the 6-hour phenylbutazone-treated group.

* $P > .05$.

† Emil Greiner Co., New York.

‡ Sterile pyrogen-free .9% NaCl (Abbott).

§ Dow Chemical Co., Midland, Mich.

TABLE I. Effect of Anti-Inflammatory Compounds in the 17-Hour Evans Blue Pleural Effusion.

Compound	Exp No.	Dose, mg/kg orally	N	Vol of pleural fluid, ml ± S.E.	% Reduction	r	λ
Acetylsalicylic acid	1	50	8	7.2 ± .21	12.2		
		150	6	6.0 ± .28	26.8	—.81	— .32
		450	8	5.0 ± .27	39.0		
	Control		5	8.2 ± .31			
	2	50	7	7.1 ± .28	10.1*		
		150	8	6.3 ± .36	20.3	—.61	— .52
		450	8	5.4 ± .36	31.7		
	Control		8	7.9 ± .29			
Phenylbutazone	1	10	8	8.4 ± .28	9.7*		
		33.3	7	7.2 ± .33	22.6	—.78	— .35
		100	7	6.2 ± .22	33.3		
	Control		8	9.3 ± .40			
	2	10	6	8.0 ± .41	13.1		
		33.3	6	6.7 ± .06	27.2	—.51	— .66
		100	8	6.1 ± .40	33.7		
	Control		7	9.2 ± .39			
Indomethacin	1	0.33	8	6.9 ± .25	10.4		
		1.00	8	6.7 ± .27	13.0	—.51	— .81
		3.33	8	5.6 ± .34	27.3		
	Control		7	7.7 ± .26			
	2	0.33	8	6.5 ± .19	8.5*		
		1.00	7	5.7 ± .32	19.7	—.56	— .64
		3.33	8	5.4 ± .27	24.0		
	Control		8	7.1 ± .27			
Mefenamic acid	1	10	8	7.7 ± .49	18.1		
		33.3	8	7.2 ± .49	23.4	—.35*	—1.14
		100	8	6.7 ± .25	28.7		
	Control		6	9.4 ± .23			
	2	10	7	6.4 ± .25	13.5		
		33.3	8	6.5 ± .21	12.2	—.29*	—1.42
		100	8	5.9 ± .23	20.3		
	Control		8	7.4 ± .26			
Flufenamic acid	1	100	8	6.5 ± .48	5.8*		
	Control		7	6.9 ± .34			
	2	100	6	7.9 ± .44	0		
	Control		7	6.9 ± .33			

* $P > .05$

terval. Table I shows acetylsalicylic acid, phenylbutazone, indomethacin and mefenamic acid by the oral route reduced the inflammatory response. Flufenamic acid was ineffective at 100 mg/kg. With the exception of mefenamic acid, the effective anti-inflammatory drugs produced reproducible dose related effects, and their indices of precision (λ) ranged from —.32 to —.81.

Flufenamic acid was further investigated measuring the volume of pleural exudate at 6 hours. This was undertaken to determine whether its duration of action was responsible for its ineffectiveness in the 17-hour experiment. Flufenamic acid was indeed active, reducing the volume of pleural exudate at low oral doses (Table II), although the reduction of pleural exudate was not dose related in two of three experiments.

In a parallel study, a comparison of the duration of action of 100 mg/kg orally of phenylbutazone and flufenamic acid was made. Fig. 2 shows that both compounds produced comparable activity at 6 and 12 hours while at 17 hours phenylbutazone continued to suppress the inflammatory response in

TABLE II. Anti-Inflammatory Activity of Flufenamic Acid in the 6-Hour Evans Blue Pleural Effusion.

Dose, mg/kg orally	Exp No.	N	Volume of pleural fluid, ml ± S.E.	% Reduction	r	λ
3.33	1	6	3.4 ± .15	10.5*		
10.		6	2.9 ± .12	23.7	—.65	— .51
33.3		6	2.8 ± .10	26.3		
Control		6	3.8 ± .27			
3.33	2	6	3.0 ± .17	30.2		
10.		6	2.8 ± .10	34.9	—.41*	— .99
33.3		6	2.5 ± .16	41.8		
Control		6	4.3 ± .20			
3.33	3	6	3.0 ± .15	23.1		
10.		6	2.8 ± .14	28.2	—.22*	—1.93
33.3		6	2.8 ± .18	28.2		
Control		6	3.9 ± .29			

* P >.05

contrast to the decrease in the effectiveness of the flufenamic acid.

Discussion. The response to the intrapleural injection of Evans Blue is unique in that fluid is initially absorbed prior to the inflammatory or effusive phase. This phenomenon has been observed by other investigators using acacia alone or together with Evans Blue(6,7).

The method of Holtkamp *et al*(1) was investigated as an assay for anti-inflammatory compounds. Since the volume of pleural exudate was low at 6 hours (Fig. 1, 2; Table II), a longer interval (17 hours), where the exudative response was consistently higher, was selected for evaluating anti-inflammatory drugs. Reproducible data obtained with phenylbutazone, acetylsalicylic acid, indomethacin and mefenamic acid is presented in Table I although no dose response effect was obtained with the last named compound. Flufenamic acid, on the other hand, was ineffective in reducing the exudative response. In the guinea pig UV-erythema(8) and carrageenin foot edema(9) assays, flufenamic acid was reported to be more active than phenylbutazone. Since these two assays span 3 and 4 hours, respectively, as compared with 18 hours for the pleural effusion assay, it was necessary to revaluate flufenamic acid measuring its activity at an earlier interval, namely 6 hours. Indeed, flufenamic acid demonstrated anti-inflammatory activity at low oral doses although the dose related response was not consistent (Table II). At this interval, the effusion is in an early stage which may contribute to the inconsistency of the results. The short duration of action of flufenamic acid was confirmed in a parallel study with phenylbutazone. At 100 mg/kg orally, flufenamic acid lasted between 12 and 17 hours while phenylbutazone, at a similar dose, persisted longer than 17 hours (Fig. 2).

Winder *et al*(8) compared the duration of anti-erythemic activity of flufenamic acid with phenylbutazone in the guinea pig UV-erythema assay. Although both compounds showed activity over a 10-hour period, their data suggested that flufenamic acid was shorter acting than phenylbutazone.

Summary. Acetylsalicylic acid, phenylbutazone, indomethacin and mefenamic acid reduced the exudative response to the intrapleural injection of Evans Blue when measurement of effect was made at 17 hours. The ineffectiveness of flufenamic acid at this time was due to its short duration of action.

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