Table II—Statistical Analysis of Aspirin Standardization Curves Modified and *Reference 1* Methods

Salicylic Acid Added, mcg.	Slope: Average	Slope: Statistical	*SD*, %	*CV*[a], %	Ratio to Control, %
Control					
0	2.02	2.04	±0.020	±0.98	
Ceric Ammonium Nitrate Treatment According to Modified Method					
0	1.90	1.89	±0.019	±1.0	93
25	1.82	1.88	±0.019	±1.0	92
100	1.87	1.88	±0.026	±1.4	92
200	1.84	1.83	±0.034	±1.8	90
Ceric Ammonium Nitrate Treatment According to Reference Method					
0	1.16	1.16	±0.020	±1.70	57
25	1.24	1.21	±0.030	±2.50	59
100	1.32	1.26	±0.022	±1.75	61
200	1.38	1.38	±0.024	±1.74	68

[a] Coefficient of variation.

enhances this "protection" considerably. Premixing of ceric ammonium nitrate and acetic acid is not recommended. The effectiveness of the ceric ammonium nitrate deteriorates rapidly under these conditions. Because the original work with ceric salts and phenols used HNO_3 (2), the latter was also tried. With 2 *N* HNO_3, recovery was almost as good as with acetic acid, but net recovery of aspirin was not the same whether or not salicylic acid was present.

Precision was evaluated by comparing the averages of the coefficients of variation of each of the four standardization curves obtained by each method (Table II). Averages and standard deviations were 1.30 ± 0.38 and 1.92 ± 0.39% for the modified and reference methods, respectively, yielding a difference between averages that was statistically significant at the 1% level (3). Reliable values by both the modified and original methods require knowledge of the salicylic acid content because blank values and aspirin standardization curves vary with salicylic acid content. While an average blank obtained with 50 mcg. salicylic acid may be used for amounts of salicylic acid up to 100 mcg. for both methods, reducing accuracy only slightly, the modified method permits using the aspirin standardization curve (calibrated with 100 mcg. salicylic acid) for a range of 0–200 mcg. salicylic acid, which is a definite advantage over the original method which requires using aspirin standardization curves appropriate to the amount of salicylic acid present (Fig. 1) (4). The modification also improves sensitivity approximately 30%.

A modification for developing fluorescence with aspirin is also presented. At a NaOH concentration of 1.2 *N*, heat was not required to convert aspirin to salicylic acid, and the fluorescence of equivalent amounts of aspirin and salicylic acid was found to be equal.

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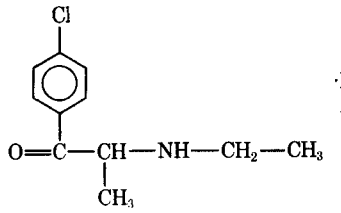
Reduced Side Effects following Administration of a New Anorectic Agent, 4′-Chloro-2-(ethylamino)propiophenone, in Timed-Release Form

EARL ROSEN, S. M. FREE, and GEORGE C. HEIL

Abstract □ Studies representing several parts of a development program for the anorexigenic SK&F 70948 are summarized. *In vitro* release rates and the results of an anorectic study in dogs demonstrated the timed-release characteristics of a 25:75 blend (25% nontimed-release pellets and 75% wax–lipid-coated pellets). The global efficacy, weight loss, and side-effect incidence of a 75- and 150-mg. timed-release formulation were studied in a multiinvestigator clinical trial; a parallel multiinvestigator clinical trial included 25- and 50-mg. t.i.d tablet regimens. In each comparison, the incidence of side effects seen with the timed-release formulation was significantly less than that seen with the t.i.d tablets ($p < 0.01$). However, the global efficacy and anorectic activity of the timed-release and t.i.d regimens did not differ significantly. These results suggested that the lower incidence of side effects following timed-release medication was related to the constant and sustained body levels of drug seen with the timed-release formulation.

Keyphrases □ 4′-Chloro-2-(ethylamino)propiophenone—reduction of side effects using timed-release form □ Timed-release dosage forms—reduction of side effects of 4′-chloro-2-(ethylamino)-propiophenone □ Anorectic agent, 4′-chloro-2-(ethylamino)propiophenone—reduction of side effects found using timed-release dosage form

During pharmaceutical development, one often attempts to modify a drug's activity in order to increase its usefulness in the treatment of disease. One such modification—prolongation of drug action—has been attempted over the years using a number of different methods with varying degrees of success. From studies comparing prolonged-action preparations with other dosage forms, it has become clear that each drug in-



SK&F 70948

volved must be considered separately and that attention must be paid to such factors as the critical dose range, the speed and duration of action, and the reliability of the therapeutic response anticipated. Most important, such considerations must be related to the advantages and disadvantages of the dosage forms being evaluated. Perhaps the most important advantage provided by prolonged-action preparations is a reduction in the variation of the drug concentration in blood and tissues that is commonly associated with multiple-dose therapy. In theory, if high peak concentrations can be avoided, the incidence and/or severity of side effects may be reduced.

The formulation of anorectic agents in prolonged-action dosage forms has become widely accepted. One such agent, SK&F 70948, a propiophenone derivative, was evaluated in clinical studies designed to provide information on global efficacy, anorectic activity, and side effects produced at different dose levels with both timed-release and tablet dosage forms. One objective of these dose range studies was to compare the incidence of side effects associated with the two dose forms; this was based on the hypothesis that, for the same total dose, the timed-release form would demonstrate a lower incidence of side effects.

In this publication, *in vitro* release data for the timed-release formulations tested are reported and an animal model found predictive of the clinical results is described.

EXPERIMENTAL

***In Vitro* Release**—Nontimed-release medicated pellets were prepared as previously reported (1, 2) and made to assay from 32 to 74% SK&F 70948. A portion of these pellets was retained, and the remaining pellets were coated with approximately 4% paraffin wax and varying increments of glyceryl stearate to prolong drug release. The release rate was determined using the rotating apparatus described in NF XIII (3).

Accurately weighed 1.0 ± 0.1 g. riffled samples were placed in 90-ml. cylindrical bottles, and 60 ml. of simulated gastric fluid USP, T.S., preheated to 37°, was added. The bottles were then placed in the 44-r.p.m. rotating apparatus submerged in a constant-temperature bath maintained at 37°. At the end of 0.5 hr., one bottle was removed from the bath, the contents were filtered through a 40-mesh metal screen, and the residue was rinsed on the screen with 25 ml. of distilled water. At the end of 1.5 hr., all the remaining bottles were removed from the bath, and the contents of the individual bottles were filtered through 40-mesh metal screens. The residues were washed free of gastric fluid with 25 ml. of distilled water, and each sample of residual pellets was returned to its respective bottle. Sixty milliliters of modified intestinal fluid[1], preheated to 37°, was added, and the bottles were replaced in the bath. After 0.5, 3, 5.5, and 8.5 hr. of additional rotation, the individual bottles were removed, the pellets were collected on 40-mesh screens, and the residual SK&F 70948 was determined as described under *Assay*.

Assay—Residual pellets were placed in a mortar, ground with 0.1 *N* aqueous H_2SO_4, and transferred to a 200-ml. volumetric flask containing about 75 ml. of 0.1 *N* H_2SO_4. The flask was shaken for 0.5 hr. and diluted to volume with the same solvent. The contents were filtered through Whatman No. 1 filter paper, with the first 15 ml. of filtrate being discarded. A 5.0-ml. aliquot of the filtrate was transferred to a 250-ml. separator containing about 25 ml. of 0.1 *N* aqueous H_2SO_4 and extracted with 30 ml. of ethyl ether. The aqueous layer was drained into a 100-ml. volumetric flask. The ether layer was washed with 30 ml. of 0.1 *N* aqueous H_2SO_4, and the latter was drained into the flask. The same solvent was used to dilute to volume. This solution was further diluted to a final concentration of about 0.015 mg. of SK&F 70948/ml. using the same solvent.

The UV absorption spectrum was recorded on a suitable spectrophotometer from 300 to 230 nm. Absorbance at the maximum (about 262 nm.) was measured and substracted from the absorbance at 290 nm. This difference was denoted ΔA. Using a spectrophotometer[2], 1st gear, 1-cm. cells, a concentration of 0.01476 mg. of SK&F 70948 (base)/ml. gave a ΔA of 1 unit.

Anorectic Effect in Dogs—Eleven adult mongrel dogs (six females and five males), weighing 10–17 kg., were preselected from a large colony of dogs that consistently ate food upon presentation and were used in this test. Control feeding studies were conducted, and it was determined that each dog would consume small portions of food (approximately 50 g.) every 30 min. over a 10-hr. period. On the day before the test, the dogs received their normal daily food offering and 2 hr. later the food cups were removed so that no food was available for 18 hr. before drug administration. Water was available *ad libitum*. On the day of the test, the dogs received either: (*a*) 48 mg./kg. of SK&F 70948 in a timed-release blend (25:75), or (*b*) three doses of 16 mg./kg. of crystalline SK&F 70948 3 hr. apart (t.i.d.). The drugs were prepared in gelatin capsules and administered in the first offering of food. The last two doses of the t.i.d. treatment were administered orally, by hand, 3 and 6 hr. after the initial dosing. Food was presented every 30 min. for 9 hr., and each offering was left in the cage for 30 min. If uneaten, the food was removed and a fresh portion was then presented. Anorexia was defined as failure to consume all of the food over two successive 30-min. periods. One week later, the animals received the second drug

Table I—*In Vitro* Release

Formulations Tested *In Vivo*	Percent Drug Released at Specified Time, hr. 0.5	2	4.5	7	10
T/R[a] blend for dog anorexia study (48 mg./kg.)	21	53	69	79	—
75-mg. T/R blend for human study	28	50	75	89	—
150-mg. T/R blend for human study	29	54	70	79	86
300-mg. T/R blend for human study	32	62	74	79	89

[a] T/R stands for timed release.

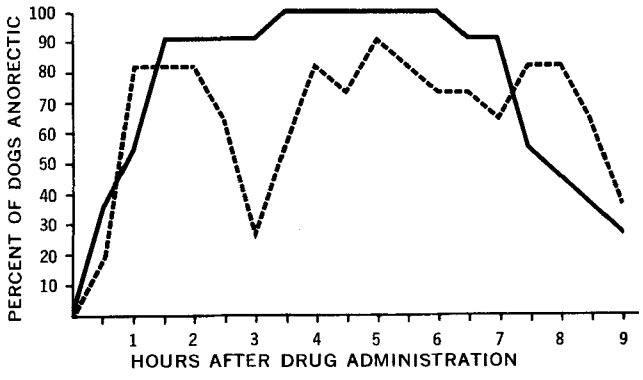


Figure 1—*Anorectic effect in dogs. Key: ——, SK&F 70948 timed-release blend, 48 mg./kg.; and - - -, SK&F 70948, 16 mg./kg. t.i.d. (at 0, 3, and 6 hr.).*

[1] Modified intestinal fluid: Dissolve 10 g. of pancreatin NF in about 700 ml. of pH 6.0 ± 0.1 phosphate buffer, and dilute to 1 l. with the same solvent.

[2] Cary model 11.

Table II—Clinical Results

	Timed-Release Formulations		
	75 mg.	150 mg.	300 mg.
Number of patients	109	112	61
Global efficacy (good and excellent results), %	30	48	50
Anorectic activity (weight loss in 8 weeks), lb.	5.2	7.4	9.3
Side effects (all), %	13	13	27
	T.I.D. Tablet		
	75 mg. (3 × 25)	150 mg. (3 × 50)	
Number of patients	125	128	
Global efficacy (good and excellent results), %	24	44	
Anorectic activity (weight loss in 8 weeks), lb.	3.6	6.1	
Side effects (all), %	28	36	

treatment so that each dog received both drug regimens in a crossover design.

Clinical Studies—In this phase of the development program, two multiinvestigator studies were carried out to measure global efficacy, anorectic activity, and side-effect incidence. These studies compared:

Study 1—75, 150, and 300 mg. of SK&F 70948 (base) in timed-release form and a standard drug.

Study 2—25- and 50-mg. tablets of SK&F 70948 (base) and a standard drug, all presented t.i.d.

Thirteen different investigators participated in each study. All followed parallel protocols where only the presentation frequency differed to accommodate the dosage form.

The patient description in the protocol indicated that patients were to be at least 10% overweight, as determined by actuarial scales, and willing to continue therapy for 8 weeks. In addition, all patients were to be treated with a 1000–2000 cal. diet and be given the same advice concerning fluid and salt restrictions and exercise programs. The timed-release form was to be taken 1 hr. before lunch; the tablets were to be taken midmorning, 2 hr. before the evening meal, and 2 hr. after the evening meal.

Each patient was interviewed and examined at 2-week intervals when the weight, blood pressure, and pulse were recorded, the anorectic effects were noted, and the presence of any side effects was marked on a case report form.

Metabolism, excretion, and biological availability studies also were conducted during this period on the plain drug and the 150-mg. timed-release form (4).

RESULTS AND DISCUSSION

Experience with the *in vitro* release patterns of preliminary formulations suggested a preparation providing 10 hr. of gradual release following a priming dose as a prototype for dog anorectic testing.

Table III—Statistical Analysis for Dose-Response Studies

Within Timed-Release Study
Global efficacy
75 mg. *versus* 150 or 300 mg.—$p < 0.05$
Anorectic activity (weight loss)
75 mg. *versus* 150 mg.—$p < 0.05$
150 mg. *versus* 300 mg.—$p < 0.05$
Side effects
75 or 150 mg. *versus* 300 mg.—$p < 0.05$
Within T.I.D. Study
Global efficacy
75 mg. *versus* 150 mg.—$p < 0.01$
Anorectic activity (weight loss)
75 mg. *versus* 150 mg.—$p < 0.05$
Side effects
75 mg. *versus* 150 mg.—N.S.

Table IV—Statistical Comparison of Clinical Findings

Global efficacy
75 mg. timed release *versus* 25 mg. t.i.d.—N.S.
150 mg. timed release *versus* 50 mg. t.i.d.—N.S.
Anorectic activity
75 mg. timed release *versus* 25 mg. t.i.d.—N.S.
150 mg. timed release *versus* 50 mg. t.i.d.—N.S.
Side effects
75 mg. timed release *versus* 25 mg. t.i.d.—$p < 0.01$
150 mg. timed release *versus* 50 mg. t.i.d.—$p < 0.01$

***In Vitro* Release**—When blends comprising 25% nontimed-release pellets and 75% wax–lipid-coated pellets were tested for *in vitro* release, the results shown in Table I were obtained. These *in vitro* data indicated that the goal had been achieved and that the formulation was suitable for *in vivo* testing.

Anorectic Effect in Dogs—Both the timed-release blend and the crystalline compound administered t.i.d. produced anorexia within 30–60 min. after administration (Fig. 1). With the timed-release blend, anorexia occurred in 91% of the dogs 1.5 hr. postdrug and in 100% of the dogs 3.5 hr. postdrug. The anorectic effect remained at the 91–100% level until 7 hr. after administration (a total of 6 hr.), when the effect began to diminish gradually. In contrast, the first dose of the t.i.d. treatment produced anorexia in 82% of the dogs in the 1st hr., falling to 27% by the 3rd hr. when the second dose was administered. One hour after the second administration (4th hr.), 82% of the dogs were again anorectic. By the 6th hr., when the third dose was administered, 73% of the dogs were still anorectic. No increase in anorectic activity was observed until 1.5 hr. after the third dose, when 82% of the dogs were affected. As with the timed-release blend treatment, the anorexia was markedly diminished by the end of the test (9th hr.). The time course of the anorexia (Fig. 1) indicates that the t.i.d. schedule produced an irregular pattern of anorectic activity while the timed-release blend produced a more sustained and consistent anorectic effect in a greater percentage of dogs. In view of these data, the timed-release preparation appeared to be a more efficient and more effective dosage form, producing more complete and sustained pharmacological effect than the t.i.d. dosage regimen.

After timed-release properties for the 25:75 blend in dogs were demonstrated successfully, the 75-, 150-, and 300-mg. formulations shown in Table I were prepared for human testing. The clinical results obtained with these regimens are summarized in Table II, and pertinent statistical comparisons are summarized in Table III.

Statistical analysis showed that a dose–response relationship did exist within each study, and this finding validated the methodology.

Because the two studies were designed in such a similar way, it was also possible to compare the results obtained in one study with those obtained in the other (Table IV).

Statistical analyses for global efficacy and side-effect incidence used the standard chi-square tests for 2 × 2 contingency tables. Weight loss averages were compared using one-sided Student's *t* tests.

In comparing these findings, it was readily apparent that the global efficacy and anorectic activity of the timed-release and t.i.d. regimens did not differ statistically, but the incidence of side effects seen after the administration of the timed-release form was significantly less than that seen after t.i.d. therapy.

One explanation for the reduced side-effect incidence can be found in the work done by Beckett and Hossie (4). These investigators studied the urinary excretion of SK&F 70948 and its metabolites following the administration of the 150-mg. timed-release form and a 50-mg. t.i.d. regimen. Their data showed that the "staircase" effect and spikes seen after the 4- and 8-hr. doses of the t.i.d. regimen were eliminated by the timed-release formulation, which maintained a steady level of unchanged drug for 16 hr. These findings led to the conclusion that the constant and sustained lower body levels of drug obtained with the timed-release formulation very likely accounted for its lower incidence of side effects.

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New Compounds: Anils

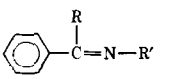
HEINO A. LUTS

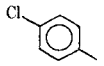
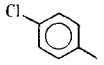
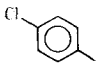
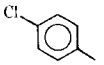
Abstract □ A series of new anils (ketimines) was synthesized by the condensation of a ketone with a desired amine.

Keyphrases □ Anils, potential antianxiety agents—synthesis by condensation of ketone with amines, preliminary screening □ Ketimines, potential antianxiety agents—synthesis, preliminary pharmacological screening □ Antianxiety agents—synthesis of new anils, preliminary screening

In search for new compounds with CNS activity, a series of anils was prepared for pharmacological evaluation (I) (Table I). These compounds were synthesized by the condensation of a ketone with a desired amine according to the procedure described in the literature (1). Preliminary pharmacological studies indicate only insignificant antianxiety properties. The complete results will be published later.

Table I—Anils



Number	R	R′	Formula	Boiling Point	Molecular Weight	n_D^{20}	Yield, %	Analysis, % Calc.	Found
I		—$CH_2CH_2N(CH_3)_2$	$C_{17}H_{19}ClN_2$	180–184°/0.6 mm.	286.78	1.5864	73	C 71.19 H 6.68 Cl 12.37 N 9.77	70.84 6.70 12.77 9.41
II		—$CH_2CH_2N(CH_2CH_3)_2$	$C_{19}H_{23}ClN_2$	160–164°/0.5 mm.	314.83	1.5735	57	C 72.48 H 7.36 Cl 11.26 N 8.89	72.39 7.44 11.50 8.68
III		—$CH_2CH_2CH_2N(CH_3)_2$	$C_{18}H_{21}ClN_2$	182–1840°/0.6 mm.	300.80	1.5790	73	C 71.87 H 7.03 Cl 11.78 N 9.31	71.63 7.03 11.69 9.41
IV		—$CH_2CH_2CH_2N(CH_2CH_3)_2$	$C_{20}H_{25}ClN_2$	164–176°/0.6 mm.	328.86	1.5678	58	C 73.04 H 7.66 Cl 10.77 N 8.51	72.76 7.59 11.01 8.33
V		—$CH_2CH_2N(CH_2CH_3)_2$	$C_{18}H_{23}N_3$	182–183°/0.3 mm.	280.37	1.5655	60	C 77.10 H 7.91 N 14.99	77.03 8.28 14.98
VI		—$CH_2CH_2CH_2N(CH_3)_2$	$C_{17}H_{21}N_3$	168–170°/0.3 mm.	267.35	1.5769	64	C 76.37 H 7.91 N 15.72	76.63 7.68 15.51
VII		—$CH_2CH_2CH_2N(CH_2CH_3)_2$	$C_{19}H_{25}H_3$	176–177°/0.3 mm.	295.41	1.5595	62	C 77.24 H 8.53 N 14.22	76.94 8.40 14.21
VIII		—$CH_2CH_2CH_2N(CH_3)_2$	$C_{16}H_{20}N_2S$	176–182°/0.6 mm.	272.39	1.5855	56	C 71.28 H 7.74 N 9.78	71.42 7.52 9.62
IX		—$CH_2CH_2N(CH_2CH_3)_2$	$C_{17}H_{22}N_2S$	172°/0.4 mm.	286.41	1.5836	55	C 70.54 H 7.40 N 10.28	70.37 7.24 9.98
X		—$CH_2CH_2CH_2N(CH_2CH_3)_2$	$C_{18}H_{24}N_2S$	182°/0.3 mm.	300.34	1.5714	53	C 71.98 H 8.05 N 9.33	71.94 8.22 9.19