

SC-18862

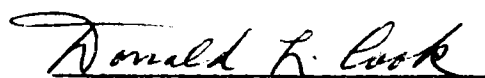
A SWEETENING AGENT

PHARMACOLOGICAL STUDIES

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INTRODUCTION

The sweetening agent, SC-18862, was subjected to a wide variety of pharmacological tests in order to delineate any possible adverse effects of the compound on the gastrointestinal system, the cardiovascular system or the central nervous system. In addition, several miscellaneous studies were carried out. Whenever feasible high oral dosages were used in order to establish high safety ratios. Since a diketopiperazine has been shown to be a degradation product of SC-18862, this compound was included in all the tests described in this report. The diketopiperazine is referred to by its code number, SC-19192, in this report.

PHARMACOLOGICAL STUDIES

A. Actions Involving the Gastrointestinal System

1. Appetite Inhibition in Rats

In this test, groups of 10 male, adult Charles River rats were housed individually and trained to eat during a two-hour period each day for four consecutive days. All rats were allowed tap water throughout the experiment. On the fifth day, one hour prior to the feeding period, each rat was weighed and given intragastrically either SC-18862, SC-19192 or the vehicle (30 per cent propylene glycol) only. One group, the fasted controls, received no treatment to ascertain the effects of fasting on body weight. One hour later all rats except the fasted controls were offered food for two hours and the grams of food consumed were measured. Twenty-four hours later the rats were again weighed. As shown in Table 1, the average weight loss for fasted controls was 20.3 g compared with only 4.1 gm for fed controls under the conditions of this experiment. Neither SC-18862 nor SC-19192 at oral doses of 200 mg/kg had any significant effect on food consumption or body weight compared with fed controls indicating that neither compound has any significant effect on appetite.

2. Effects on Gastric Secretion in Rats

SC-18862 and SC-19192 were tested for their effects on gastric secretion in rats by a modification of the method of Shay et al. (1). Briefly, male Sprague-Dawley rats supplied by Charles River weighing between 175 and 225 g and previously fasted for 48 hours were

Table 1
Effects of Oral SC-18862 and SC-19192
on Appetite in Rats

Treatment	Dose mg/kg	No. of Rats	Food Consumption (g) mean \pm S.E.	Weight Loss (g) mean \pm S.E.
Fed Controls	--	10	11.9 \pm 1.4	4.1 \pm 1.3
Fasted Controls	--	10	0	20.3 \pm 0.9*
SC-18862	200	10	12.8 \pm 1.4	4.4 \pm 1.2
SC-19192	200	10	11.9 \pm 0.7	5.6 \pm 0.9

* Significantly different from other treatments ($P < 0.05$).

anesthetized with ether and subjected to pyloric ligation. Immediately following ligation, the test compounds suspended in 1 ml of distilled water were administered intragastrically. A group of rats serving as controls were given 1 ml of distilled water only. Five hours later the stomachs were removed, gastric contents measured and centrifuged. Acid concentration was determined by titrating aliquots of the centrifuged gastric juice with 0.1 N NaOH to pH 7.0. Proteolytic activity of the gastric juice was determined by the method of Anson and Mirsky (2) and expressed as optical density of the final filtrate. The results of this test are summarized in Table 2. As shown, neither SC-18862 nor SC-19192 at oral doses of 50 mg/rat (approximately 250 mg/kg) had any significant effects on gastric juice volume, acidity or proteolytic activity.

3. Pepsin Inhibition In Vitro

The in vitro anti-pepsin activity of SC-18862 and SC-19192 was determined by a modification of the hemoglobin method of Anson and Mirsky (2). In brief, the method consists of the incubation of bovine pepsin with bovine hemoglobin in the presence of the test compound at a concentration of 143 µg/ml. After a two-hour incubation period at 37°C., the reaction was stopped by the addition of 20 per cent perchloric acid and the contents of each tube allowed to flocculate. The samples were then filtered, the filtrate diluted one part to ten parts with sodium acetate buffer (pH 5) and their optical densities determined at 275 mµ. The optical densities so obtained were compared with similarly obtained filtrates in which the test compounds were added at the end of the incubation period (controls). The results of these tests are

Table 2
Effects of SC-18862 and SC-19192 on Gastric Secretion in Pyloric-Ligated Rats

Treatment	Oral Dose mg/rat	No. of Rats	Gastric Secretion				Proteolytic Activity O.D. @ 275 mu mean \pm S.E.
			Volume ml/5 hrs mean \pm S.E.	Acidity		mEq/5 hrs mean \pm S.E.	
				mEq/L mean \pm S.E.	mEq/5 hrs mean \pm S.E.		
Controls	--	6	6.9 \pm 0.9	106 \pm 8.3	0.75 \pm 0.10	0.547 \pm 0.009	
SC-18862	50	6	5.2 \pm 0.9	117 \pm 5.0	0.66 \pm 0.40	0.569 \pm 0.008	
SC-19192	50	6	6.7 \pm 0.4	98 \pm 3.3	0.60 \pm 0.10	0.530 \pm 0.005	

summarized in Table 3. As shown, SC-18862 and SC-19192 did not inhibit pepsin. A known pepsin inhibitor, amylopectin sulfate, is also included in the table. By way of comparison, this agent significantly inhibited the proteolytic activity of pepsin in this test.

4. Pancreatic Lipase Inhibition In Vitro

The hydrolysis of triglycerides by pancreatic lipase in vitro results in the liberation of free fatty acids. The amount of titratable acid appearing after two hours of incubation provides a measure of the rate of hydrolysis. An active compound would significantly decrease fatty acid formation. In this assay, SC-18862 and SC-19192 at concentrations of 1.25 mg/ml produced no significant decrease in lipase activity (Table 4).

5. Effects on Gastric Ulceration in Rats

The inhibitory effects of SC-18862 and SC-19192 on gastric ulceration was measured by a modification of the method of Shay et al. (1). Male Charles River rats, weighing between 200 and 250 grams each, were lightly anesthetized with ether and subjected to pyloric ligation. They were then immediately given compound or vehicle intragastrically. Seventeen and one-half hours later the stomachs were surgically removed and examined under 5x magnification. The numbers of ulcers occurring in the nonsecretory portion of each stomach were counted in four groups according to size (<2 mm, 2-4 mm and >8 mm). Each rat was given a score, Z, a weighted average of the logarithms of the ulcer

Table 3
In Vitro Test for Pepsin Inhibition

Treatment	Concentration μg/ml	No. of Tests	Optical Density	
			Control ¹ mean ± S.E.	Treat ² mean ± S.E.
SC-18862	143	8	0.384 ± 0.003	0.381 ± 0.004
SC-19192	143	2	0.372 ± 0.001	0.376 ± 0.004
Amylopectin Sulfate	143	8	0.394 ± 0.012	0.324 ± 0.011*

¹ Test compound added at the end of the 2-hour incubation period.

² Test compound added at the beginning of the 2-hour incubation period.

* Significant decrease from control mean (P < 0.05).

Table 4

In Vitro Test for Pancreatic Lipase Inhibition*

Treatment	Concentration mg/ml	Duplicates	Mean ml of 0.1 N NaOH ¹	
			Control ²	Treat ³
SC-18862	1.25	2	2.97	2.99
SC-19192	1.25	2	2.72	2.77

* A difference of > 0.39 ml of 0.1 N NaOH is significantly different from control based on long term studies ($P < 0.05$).

¹ ml needed to titrate 14 ml of emulsified triglyceride (olive oil) containing 0.4 mg/ml of pancreatic lipase.

² Compound added to the emulsion after the 2-hour incubation period.

³ Compound present in the emulsion during the 2-hour incubation period.

counts in the four size groups.* Each treatment group was comprised of six rats. Based on long term studies a decrease in the mean Z score $\bar{Z} > 31$ from concurrent controls is regarded as a significant change ($P < 0.05$). As shown in Table 5, SC-18862 and SC-19192 at doses of 50 mg/rat were inactive in this test. Neither compound significantly inhibited or increased the severity of gastric ulceration. In contrast, Pro-Banthine, a known anti-ulcer agent, significantly inhibited ulceration in this test.

B. Actions Involving the Cardiovascular System

1. Effects on Blood Pressure in Anesthetized Dogs Following Intravenous Administration

The effects of SC-18862 and SC-19192 on arterial blood pressure were determined in two mongrel dogs anesthetized with sodium pentobarbital. Blood pressure was monitored continuously from a femoral artery in each dog. The compounds were dissolved in propylene glycol and injected intravenously via a femoral vein. SC-18862 at doses of 0.1 and 1.0 mg/kg had no effect on blood pressure in either dog. A dose of 5 mg/kg induced only a slight transient lowering of blood pressure in one dog and no effect in the other dog. SC-19192 had no effect on blood pressure at doses of 0.1, 1.0 and 5 mg/kg.

* The formula that has been found approximately optimal by discriminant function analysis is: $Z = 20.00 \log(N_1 + 1) + 0.22 \log(N_2 + 1) + 46.76 \log(N_3 + 1) + 6.11 \log(N_4 + 1)$; where $N_1..N_4$ are the observed ulcer counts of the increasing size group.

Table 5
Effects of SC-18862 and SC-19192 on Gastric Ulceration
Induced in Rats by Pyloric Ligation

Treatment	Oral Dose mg/rat	No. of Rats	Ulcer Score* \bar{Z}
Controls	--	6	79.8
SC-18862	50	6	70.2
Controls	--	6	71.9
SC-19192	50	6	45.4
Controls	--	6	78.6
Pro-Banthine	0.5	6	13.8**

* See text for explanation of \bar{Z} score.

** Significantly different from concurrent controls.

2. Effects on Blood Pressure and Heart Rate Following Oral Administration in Unanesthetized Normotensive Dogs

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Blood pressure was recorded via a surgically implanted aortic cannula. After surgery the animals were trained to lie quietly on a pad within a sound attenuating environmental chamber to isolate the animals from outside disturbances. Control readings were made periodically following surgery to confirm that the cardiovascular parameters had stabilized before the animals were used experimentally. Systolic, diastolic and mean arterial pressure were recorded on an oscillographic recorder (Brush-Model 440) using a miniature pressure transducer (Micron-Model MP-15). Heart rate was derived from the blood pressure tracing. Blood pressure and heart rate values were determined at 5 minute intervals during a 30 minute control period before administration of the compound. These values were averaged, and the average with the range was used as a measure of the animal's pretreatment state. SC-18862 and SC-19192 were administered in capsule form at doses of 0 (placebo), 100 and 200 mg/kg to each of 2 dogs. Measurements were made at 10, 20, 30, 40, 50, 60, 120, 180, 240 minutes and 24 hours after treatment. These values are listed in Tables 6-17 and are expressed additionally as a per cent change from the calculated control means.

Results - SC-18862

Dog #G23 male. Data are shown in Tables 6-8. Table 6 illustrates the stability of this animal after placebo administration. Systolic and diastolic pressures after treatment show only single values

Table 6

The Effects of a Placebo on Blood Pressure and Heart Rate
of the Unanesthetized Dog.
(No. G23 Male Wt. 13.7 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	111	62	84	60
- 25	113	67	88	78
- 20	118	68	92	54
- 15	111	62	86	60
- 10	110	64	84	60
- 5	108	69	92	102
0	112	64	87	66

<u>Pretreatment Means and Ranges</u>				
	112 (108-118)	65 (62-69)	88 (84-92)	69 (54-102)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	110 -2	69 +6	89 +2	66 -4
+ 20	113 +1	66 +2	88 0	72 +5
+ 30	110 +1	*76 +17	84 -4	60 -13
+ 40	108 -3	*60 -8	*78 -11	54 -21
+ 50	*121 +8	*73 +12	90 +3	78 +14
+ 60	116 +4	68 +4	86 -2	72 +5
+120	115 +3	66 +1	86 -2	60 -13
+180	114 +2	60 -8	*96 +10	66 -4
+240	108 -4	66 +1	84 -4	60 -13
+24 hrs.	114 +2	66 +1	86 -2	72 +5

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pre-treatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 7

The Effects of SC-18862 100 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. G23 Male Wt. 13.3 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	122	73	90	60
- 25	149	86	100	114
- 20	140	70	98	96
- 15	113	72	95	66
- 10	124	70	95	72
- 5	136	76	98	96
0	114	65	98	72

<u>Pretreatment Means and Ranges</u>				
	128 (113-149)	73 (65-86)	96 (90-100)	82 (60-114)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	116 -10	*60 -18	*80 -17	60 -27
+ 20	114 -11	*64 -12	*88 -9	60 -27
+ 30	116 -10	*64 -12	96 0	72 -12
+ 40	118 -8	*64 -12	*84 -13	*54 -34
+ 50	*108 -16	*56 -23	*78 -19	*54 -34
+ 60	*106 -17	*58 -21	*86 -11	*54 -34
+120	114 -11	*64 -12	*80 -17	*54 -34
+180	*112 -13	*62 -15	*78 -19	*48 -42
+240	*108 -16	*64 -13	*78 -19	*54 -34
+24 hrs.	*110 -14	*63 -14	*78 -19	*54 -34

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 8

The Effects of SC-18862 200 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. G23 Male Wt. 13.8 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	123	74	90	60
- 25	112	60	86	60
- 20	108	73	84	66
- 15	108	64	84	72
- 10	114	66	82	66
- 5	110	57	80	60
0	105	74	85	54

<u>Pretreatment Means and Ranges</u>				
	111 (105-123)	67 (57-74)	84 (80-90)	63 (54-72)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	106 -5	67 0	88 +4	60 -4
+ 20	122 +9	71 +6	*95 +12	54 -14
+ 30	*127 +14	*76 +14	*92 +9	60 -4
+ 40	120 +8	74 +11	89 +5	60 -4
+ 50	121 +9	74 +11	90 +7	54 -14
+ 60	120 +8	66 -1	88 +4	66 +5
+120	121 +9	72 +8	89 +5	72 +15
+180	*126 +13	*75 +12	*94 +11	60 -4
+240	118 +6	70 +5	*91 +8	66 +5
+24 hrs.	113 +1	58 -13	*76 -10	60 -4

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

outside the pretreatment range (i.e. +8% and +17% respectively). Mean arterial pressure remained near control throughout the course of the post-treatment period with maximum changes of only -11% and +10% seen at +40 and +180 minutes respectively.

After a dose of 100 mg/kg of SC-18862 (See Table 7) all blood pressure and heart rate readings were depressed below control range but showed remarkable stability. Systolic pressure was maximally depressed at +60 minutes (-17%); diastolic at +50 minutes (-23%) and mean pressure at +180, +240 and 24 hours (-19%). Heart rate was depressed proportionally greater, showing a maximum depression at +180 minutes of -42%. It should be noted that pretreatment values for both blood and heart rate were appreciably higher in this test than they were in other tests carried out in this same dog (Tables 6 and 8). Table 8 displays the results in the same animal after 200 mg/kg of SC-18862. The control ranges of all parameters were relatively narrow. After treatment systolic pressure was generally elevated, and showed a maximal (+14%) rise after 30 minutes. Diastolic pressure changes followed a similar elevated pattern and a maximum elevation of +14% after 30 minutes. Mean pressure also showed a maximum (+12%) elevation at +30 minutes. In contrast to the pressure values seen, heart rate responses were mixed, with all values, however, falling within the pretreatment range (i.e. between -14% to +15%).

Dog #F41 female. Data from this animal are shown in Tables 9-11. As was seen in Table 9 this animal showed more variability following the placebo than the previous animal. Systolic pressure was generally depressed and showed a maximum decrease (-20%) after 50 minutes. Diastolic pressure likewise showed a maximum depression at +50 minutes of -13%. All other values fell within the pretreatment

Table 9

The Effects of a Placebo on Blood Pressure and Heart Rate
of the Unanesthetized Dog.
(No. F41 Female Wt. 5.85 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	162	68	93	90
- 25	164	66	88	84
- 20	172	68	100	108
- 15	166	66	92	96
- 10	172	78	105	114
- 5	152	61	87	90
0	154	62	85	84

<u>Pretreatment Means and Ranges</u>				
	163 (152-172)	67 (61-78)	93 (85-105)	95 (84-114)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	160 -2	67 0	97 +4	114 +20
+ 20	*148 -9	63 -6	88 -5	102 +7
+ 30	163 0	61 -9	94 +1	102 +7
+ 40	153 -6	68 +1	90 -3	108 +14
+ 50	*130 -20	*58 -13	*82 -12	114 +20
+ 60	155 -5	63 -6	90 -3	108 +14
+120	159 -3	69 +3	98 +6	* 78 -18
+180	170 +4	76 +13	104 +12	* 78 -18
+240	152 -7	64 -4	96 +3	88 -8
+24 hrs.	152 -7	63 -6	89 -4	90 -5

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 10

The Effects of SC-18862 100 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. F41 Female Wt. 6.3 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	149	70	97	96
- 25	150	68	100	90
- 20	164	80	96	114
- 15	154	77	92	90
- 10	148	66	88	84
- 5	138	60	90	84
0	154	74	98	84

<u>Pretreatment Means and Ranges</u>									
151 (138-164)			71 (60-80)		94 (88-100)		92 (84-114)		
<hr/>									
<u>Post-treatment:</u>	(1)		(2)		(3)		(4)		
+ 10	*166	+10	77	+9	*110	+16	102	+11	
+ 20	*169	+12	*84	+19	*114	+21	*116	+26	
+ 30	*173	+15	*100	+41	*116	+23	108	+18	
+ 40	*166	+10	*102	+44	*119	+26	*132	+44	
+ 50	*179	+18	* 90	+27	*106	+12	102	+11	
+ 60	*175	+16	* 81	+14	*108	+14	84	-8	
+120	158	+5	74	+5	*108	+14	84	-8	
+180	152	+1	*84	+19	*105	+11	*132	+44	
+240	162	+7	73	+3	*104	+10	92	-2	
+24 hrs.	142	-6	61	-14	92	-3	84	-8	

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 11

The Effects of SC-18862 200 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. F41 Female Wt. 5.9 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	160	96	122	138
- 25	149	88	110	90
- 20	127	80	98	102
- 15	128	78	98	132
- 10	116	78	94	126
- 5	92	70	84	108
0	155	61	90	102

Pretreatment Means and Ranges

	132 (92-160)	79 (61-96)	99 (84-122)	114 (90-138)
<u>Post-treatment:</u>				
(1)		(2)	(3)	(4)
+ 10	*172 +30	68 -14	100 +1	126 +10
+ 20	*162 +22	74 -6	89 -10	120 +5
+ 30	150 +13	78 -1	101 +2	138 +21
+ 40	*161 +22	71 -10	106 +7	114 0
+ 50	*165 +25	82 +4	104 +5	126 +10
+ 60	150 +13	74 -6	98 -1	108 -5
+120	136 +3	72 -9	99 0	96 -16
+180	136 +3	74 -6	96 -3	90 -21
+240	132 0	*60 -24	*80 -20	90 -21
+24 hrs.	132 0	*56 -29	87 -12	*84 -26

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

range. Mean pressure also reached a maximum depression at +50 minutes of -12% with all other values in the pretreatment range. Heart rate was generally increased after placebo, reaching a maximum of +20% at +50 minutes which was still within the pretreatment range. At +120 and +180 minutes the maximal decrease of -18% was observed. These values fell below the control range. After 100 mg/kg of SC-18862 (Table 10) almost all values recorded were elevated above the control means. This is in contrast to the previous animal. Almost all pressure values were above the control range. Systolic pressure reached a maximum of +18% at +50 minutes. Diastolic maximum of +44% was reached at +40 minutes. Heart rate following treatment showed widest variation (i.e. +44% at +40 and +180 minutes). Following a dose of 200 mg/kg of SC-18862 the changes did not appear to be dose related (Table 11). Systolic pressure was initially elevated outside the pretreatment range and reached a maximum +30% elevation 10 minutes after drug administration. Diastolic pressure tended toward a depression from the control mean but the parameters remained within the pretreatment range until +240 minutes. The 4 hour and 24 hour readings were lowered -24 and -29% respectively after treatment. Mean arterial pressure remained within the control range with the exception of the +240 minute reading which showed a -20% change from control. Heart rate changes were variable but within the control range also except at the 24 hour reading which was depressed 26%.

Results - SC-19192

Dog #G22 male. Data from this dog are shown in Tables 12-14.

After a placebo capsule was given, the animal showed considerable variation.

Table 12

The Effects of a Placebo on Blood Pressure and Heart Rate
of the Unanesthetized Dog.
(No. G22 Male Wt. 8.0 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	144	74	86	54
- 25	140	72	84	66
- 20	142	68	90	54
- 15	145	78	97	120
- 10	150	74	94	72
- 5	142	74	88	72
0	152	82	106	96

<u>Pretreatment Means and Ranges</u>				
	145 (140-152)	75 (68-82)	92 (84-106)	76 (54-120)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	148 +2	78 +5	98 +6	72 -6
+ 20	150 +3	79 +6	96 +4	78 +2
+ 30	*153 +6	*92 +23	102 +11	78 +2
+ 40	148 +2	80 +7	100 +9	78 +2
+ 50	*158 +9	*86 +15	104 +13	90 +18
+ 60	*161 +11	*98 +31	*114 +24	102 +34
+120	*171 +18	*104 +40	*120 +30	*126 +65
+180	*139 -4	74 -1	100 +9	90 +18
+240	146 +1	*87 +17	*108 +17	108 +42
+24 hrs.	148 +2	*90 +21	*112 +22	120 +57

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 13

The Effects of SC-19192 100 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. G22 Male Wt. 7.65 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	151	81	100	84
- 25	148	76	98	78
- 20	149	82	94	72
- 15	161	86	100	66
- 10	150	81	98	78
- 5	170	76	111	114
0	154	74	92	72

<u>Pretreatment Means and Ranges</u>				
	155 (148-170)	80 (76-86)	99 (92-111)	81 (66-114)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	161 +4	86 +8	98 -1	78 -3
+ 20	154 0	80 0	96 -3	66 -18
+ 30	160 +3	*93 +17	108 +9	96 +19
+ 40	164 +6	*99 +24	102 +3	66 -18
+ 50	*138 -11	*72 -10	96 -3	66 -18
+ 60	156 +1	77 -3	98 -1	66 -18
+120	160 +3	*90 +13	109 +10	90 +12
+180	*142 -8	82 +3	102 +3	90 +12
+240	152 -2	*87 +9	110 +11	96 +19
+24 hrs.	153 -1	*88 +10	109 +10	96 +19

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 14

The Effects of SC-19192 200 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. G22 Male Wt. 7.9 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	178	102	116	57
- 25	158	84	103	56
- 20	158	87	112	59
- 15	166	95	114	65
- 10	167	96	114	56
- 5	166	95	110	56
0	165	92	110	56

Pretreatment Means and Ranges

	165 (158-178)	93 (84-102)	111 (103-116)	58 (56-58)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	*142 -14	86 -8	*100 -10	57 -1
+ 20	*157 - 5	*76 -18	103 -7	*96 +66
+ 30	*157 - 5	*80 -14	*100 -10	*84 +45
+ 40	168 + 2	94 + 1	*100 -10	*101 +75
+ 50	*150 - 9	*76 -18	*96 -14	*92 +59
+ 60	*156 - 6	*78 -16	*96 -14	*86 +49
+120	171 + 3	92 - 1	112 + 1	*90 +56
+180	*153 - 8	90 - 3	114 + 2	*54 - 7
+240	*154 - 7	95 + 2	112 + 1	*101 +75
+24 hrs.	158 - 6	*80 -14	114 + 2	*96 +66

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

The overwhelming trend following treatment was in a positive direction with only single values of systolic and diastolic pressure being below the control average. Systolic pressure after 180 minutes was decreased (4%) from the control pretreatment mean. Systolic pressure showed a maximum increase (18%) at +120 minutes. Diastolic pressure showed a similar time pattern of change. It also reached its maximum (+40%) after 120 minutes. Mean arterial pressure followed both systolic and diastolic pressure and displayed a maximum change (+30%) after 120 minutes. These maxima were all above the pretreat range. Heart rate was elevated generally after placebo and demonstrated the greatest percentage change of all the variables (i.e. +65% at 120 minutes). As can be seen in Table 13, treatment of this animal with 100 mg/kg of SC-19192 lowered the experimental variation seen after placebo. Systolic pressure exhibited only two values outside the pretreatment range, with the maximum being a -11% decrease at +50 minutes. Diastolic pressure showed a mixed response of increased and decreased values falling between -10% (+50 minutes) and +24% (+40 minutes). Mean arterial pressure and heart rate remained with the pretreatment range throughout the experiment. Respectively these parameters varied from -3 to +11% and -18 to +19% from the control mean. Table 14 contains the values seen after treatment with 200 mg/kg of wC-19192. After this dose most of the pressure parameters were depressed below the pretreatment range the remainder of the experiment. Systolic pressure reached a minimum values of -14% at +10 minutes. Diastolic pressure reached -18% at +50 minutes while the mean pressure showed a minimum of -14% at +50 and 60 minutes. Heart rate, in contrast, showed excessive elevation throughout the experiment reaching a maximum

increase of +75% at +240 minutes.

Dog #F29 male. Data from this animal are displayed in Tables 15-17. Unlike the results from the previous animal the placebo administration was followed by changes in both the positive and negative direction from the control means. Values ranged above and below the pre-treatment range. Systolic pressure varied from -19% (240 minutes) to +18% (50 minutes) of the control mean. Diastolic pressure showed even more variation, from -22% (240 minutes) to +22% (50 minutes). Mean pressure mirrored the above changes with variation extending from -20% at 24 hours to +12% at 50 minutes. Heart rate after placebo was also erratic demonstrating changes from -18% at 24 hours to +34% at 50 minutes. Following the 100 mg/kg dose of SC-19192, the animal showed less variation (Table 16) but the pressure responses were uniformly depressed below the control range. Systolic, diastolic and mean pressure declined steadily to a maximal drop at 24 hours of -24%, -30% and -25% respectively. Heart rate reacted in an opposite manner, and was consistently elevated.

A maximum increase of +64% was observed at +120 minutes. Additionally, values of +42% were seen at 20, 30 and 240 minutes after treatment. Changes seen after 200 mg/kg of SC-19192 (Table 17) failed to show a progression of the effects noted after 100 mg/kg in this dog. Blood pressure values were higher than after the lower dose and did not show the extreme changes from the control mean. Systolic pressure values oscillated between -8% (50 minutes) and +16% (20 minutes). Diastolic pressure also varied about the control mean with values of -9% (60 minutes)

Table 15

The Effects of a Placebo on Blood Pressure and Heart Rate
of the Unanesthetized Dog.
(No. F29 Male Wt. 11.5 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	122	82	95	84
- 25	130	86	98	90
- 20	126	82	104	84
- 15	122	80	96	78
- 10	124	80	104	72
- 5	124	86	102	84
0	120	78	98	72

<u>Pretreatment Means and Ranges</u>				
	124 (120-130)	82 (78-86)	100 (95-104)	81 (72-90)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	*114 -8	*74 -10	* 92 -8	72 -11
+ 20	128 +3	*92 +12	98 -2	78 -3
+ 30	*114 -8	*76 -7	*92 -8	*66 -18
+ 40	120 -3	78 -5	*93 -7	72 -11
+ 50	*146 -18	*100 +22	*111 +12	*108 +34
+ 60	*132 +6	85 +4	104 +4	90 +12
+120	*110 -11	*68 -17	*86 -14	*66 -18
+180	*116 -6	78 -5	*88 -12	84 +4
+240	*100 -19	*64 -22	*87 -13	72 -11
+24 hrs.	*108 -13	*67 -18	*80 -20	*66 -18

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 16

The Effects of SC-19192 100 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. F29 Male Wt. 11.7 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	135	78	106	60
- 25	134	85	100	54
- 20	138	90	104	54
- 15	150	92	102	54
- 10	141	93	106	54
- 5	148	94	104	54
0	141	90	108	54

Pretreatment Means and Ranges

	141 (134-150)	89 (78-94)	104 (100-108)	55 (54-60)
<u>Post-treatment:</u>				
	(1)	(2)	(3)	(4)
+ 10	*120 -32	*72 -19	*94 -10	54 -2
+ 20	*130 -8	86 -3	*97 -7	*78 +42
+ 30	*132 -6	84 -5	*100 -4	*78 +42
+ 40	*124 -12	80 -10	*93 -11	54 -2
+ 50	*124 -12	86 -3	*93 -11	*72 +31
+ 60	*128 -9	82 -8	*96 -8	60 +9
+120	*125 -17	*72 -19	108 -14	*90 +64
+180	*122 -14	*76 -14	*86 -18	*72 +31
+240	*114 -19	*70 -21	*82 -21	*78 +42
+24 hrs.	*107 -24	*62 -30	*78 -25	54 -2

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

Table 17

The Effects of SC-19192 200 mg/kg I.G. on Blood Pressure
and Heart Rate of the Unanesthetized Dog.
(No. F29 Male Wt. 11.6 kg)

Time (min)	Arterial Blood Pressure			Heart Rate (beats/min)
	Systolic (mm Hg)	Diastolic (mm Hg)	Mean (mm Hg)	
<u>Pretreatment:</u>				
- 30	92	61	72	48
- 25	96	54	64	48
- 20	94	52	66	48
- 15	95	54	70	42
- 10	106	61	70	42
- 5	102	58	73	42
0	102	60	77	42

<u>Pretreatment Means and Ranges</u>				
	98 (92-106)	57 (52-61)	70 (64-77)	45 (42-48)
<u>Post-treatment:</u>	(1)	(2)	(3)	(4)
+ 10	*112 +14	56 -2	68 -3	48 +8
+ 20	*114 +16	*67 +17	*84 +20	*54 +21
+ 30	*107 +9	*62 +8	71 +1	42 -5
+ 40	94 -4	56 -2	*79 +12	*54 +21
+ 50	*90 -8	*66 +16	68 -3	*72 +62
+ 60	93 -5	52 -9	70 0	*84 +88
+120	96 -2	60 +5	76 +8	*78 +75
+180	97 -1	*66 +16	*81 +15	*66 +48
+240	96 -2	60 +5	*81 +15	*96 +115
+24 hrs.	96 -2	58 +2	72 +2	*66 +48

* Indicates value is outside control range.

Columns (1), (2), (3) and (4) show percentage deviation from pretreatment mean values for systolic, diastolic, and mean blood pressure, and heart rate.

and +17% (20 minutes) of the pretreatment mean. Heart rate progressively increased throughout the experiment and reached a maximum of 115% above the control mean after 4 hours.

Summary

The cardiovascular effects of placebo, SC-18862 and SC-19192 were investigated in four normal unanesthetized dogs after oral administration. No consistent effects of either compound on blood pressure at the 100 or 200 mg/kg dose were seen between individual dogs. No dose related blood pressure effects could be seen in individual animals with either compound. Changes in one animal following a specific dose were generally in an opposite direction from the other dose level. Heart rate changes generally followed the same lack of pattern. Day to day variations in pretreatment measurements for each animal were approximately as great as the changes observed following treatment. No side effects were observed during the course of the experiments or during the interim periods between treatments.

3. Inhibition of the Pressor Response to Angiotensin in Rats

The ability of SC-18862 or SC-19192 to block the vasopressor activity of angiotensin was tested by a modification of the procedure described by Pickens et al. (3). Adult male Charles River rats were anesthetized by intraperitoneal injection of sodium pentobarbital. Cardiovascular reflexes were blocked with atropine and pentolinium given subcutaneously. A femoral vein for intravenous injections and a femoral artery for blood pressure were cannulated. After blood pressure

had stabilized, five consecutive doses of 0.01 μ g of synthetic angiotensin were administered intravenously at three-minute intervals. Three minutes after the last injection of angiotensin, SC-18862 or SC-19192 was administered intravenously at a dose of 10 mg/kg. After 15 minutes, the five consecutive doses of angiotensin were repeated. The mean of the pressor responses to the pretreatment doses of angiotensin was computed and compared to the mean of the responses after compound administration. The results of these tests are summarized in Table 18. Neither SC-18862 nor SC-19192 had any significant effect on the pressor responses to angiotensin following intravenous administration of 10 mg/kg. On the other hand, hydralazine, a known antihypertensive agent, markedly inhibited angiotensin following an intravenous dose of 10 mg/kg.

4. Antiarrhythmic Activity Using the Isolated Rabbit Heart

The ability of SC-18862 to affect aconitine-induced ventricular arrhythmia in the isolated rabbit heart was determined using a modification of the Langendorff procedure described by Lucchesi (4). Similar tests were carried out with quinidine sulfate as a standard antiarrhythmic agent. A compound is considered active in this test if it reduces the ventricular rate, elevated by aconitine, by 50%. As shown in Table 19, both SC-18862 and SC-19192 were inactive in reversing the effects of aconitine in two experiments at concentrations of 10, 20 and 40 mg/L. On the other hand, quinidine sulfate was effective at 10 and 40 mg/L.

Table 18
 Test for Antagonism of the Pressor Response
 to Angiotensin in Rats

Treatment	Dose mg/kg i.v.	No. of Rats	Mean Angiotensin-Induced Increase in Mean Blood Pressure (mmHg)	
			Pretreatment mean \pm S.E.	Post-treatment mean \pm S.E.
SC-18862	10	2	15.2 \pm 1.4	16.4 \pm 1.8
SC-19192	10	2	33.8 \pm 9.4	38.2 \pm 11.0
Hydralazine	10	2	40.1 \pm 10.1	9.8 \pm 1.0*

* Significantly different from pretreatment response ($P < 0.05$).

Table 19

Effects of SC-18862 and SC-19192 on Aconitine-Induced Arrhythmia in the

Isolated Rabbit Heart

Treatment	Rabbit No.	Control	Aconitine ¹	10 mg/l ²		20 mg/l		40 mg/l	
				5 min	10 min ³	5 min	10 min	5 min	10 min
SC-18862	1	90	225	230	230	250	250	230	220
	2	110	250	260	260	255	250	240	235
SC-19192	1	85	210	210	210	200	200	190	190
	2	110	220	220	190	190	190	190	180
Quinidine SO ₄	1	100	200	210	75	100	110	50	50
	2	80	210	215	140	140	110	120	80

¹ Heart rate ten minutes after injection of 0.05 ml of 0.1% aconitine nitrate into the apex of the perfused heart.

² mg per liter of perfusion solution.

³ minutes after addition of treatment.

5. Effects on Blood Coagulation In Vitro

The effects of SC-18862 and SC-19192 on coagulation time of whole blood were determined by the method of Lee and White (5). Saline solutions of the two compounds were added to freshly drawn rabbit blood and incubated at 37°C. Coagulation time was compared to that obtained with U.S.P. Heparin. The average coagulation time of control blood, derived from 24 determinations, was found to be 3.3 minutes (range: 1-6 minutes); therefore, coagulation times of 7 minutes or longer were considered active. As shown in Table 20, both SC-18862 and SC-19192 were inactive at concentrations greater than 33 times the minimal effective concentration of Heparin.

C. Actions Involving the Central Nervous System

1. General Effects in Mice

Groups of four mice each were injected intraperitoneally or subcutaneously with doses of either SC-18862 or SC-19192 ranging from 5 to 320 mg/kg. Observations and tests were carried out with each mouse just prior to compound administration and at 1/2, 1, 2, 3 and 4 hours after treatment. At each interval, the spontaneous and elicited behavior of each mouse was rated along a continuum which included marked depression through normality to marked excitation, with scores for each mouse ranging from a maximum of 5 points for either depression or excitation. Tests for locomotor ataxia were carried out at each of the time intervals by placing each mouse on a horizontally suspended 1/2 inch aluminum rod and observing his

Table 20
In Vitro Anticoagulant Activity of
 SC-18862 and SC-19192

Test Substance	Clotting Time in Minutes at Concentrations (mg/ml rabbit blood) of:					
	0.1	0.05	0.025	0.013	0.006	0.003
SC-18862	3	3	4	--	--	--
SC-19192	4	4	5	--	--	--
Heparin, U.S.P.	>300	75	45	15	12	10

locomotor-postural performance for approximately 30 seconds. Ataxia was rated semiquantitatively on a 4 point scale which included no ataxia (0), mild to moderate (1, 2) to extreme ataxia (3) with the endpoint being simply the observer's judgment as to the quality of performance. The results of these tests are summarized in Tables 21 and 22. As shown, neither SC-18862 nor SC-19192 caused excitation or depression at any of the doses used. Very slight ataxia was recorded for SC-18862 at intraperitoneal doses of 20, 40, 80 and 320 mg/kg and subcutaneous doses of 40, 80 and 320 mg/kg. Similar observations were recorded for SC-19192 following intraperitoneal doses of 5, 20, 40, 80 and 320 mg/kg and subcutaneous doses of 40 and 320 mg/kg. This slight ataxia was not considered biologically significant due to the lack of relationship to dosage. Furthermore, a more objective measurement of locomotor effects was later carried out using the rotating rod technique (see section C. 4.) and SC-18862 and SC-19192 were found to be inactive following oral doses of 200 mg/kg.

2. Antidepressant Activity in Mice

SC-18862 and SC-19192 were tested for antidepressant activity by determining their ability to antagonize the ptosis response in mice following injection of RO 4-1284, a synthetic benzoquinolizine with reserpine-like activity. One hour following intragastric administration of the test compounds to groups of 10 mice each, RO 4-1284 was administered intraperitoneally at a dose of 20 mg/kg. This dose of RO 4-1284 induces complete ptosis in control mice. Ptosis is graded by the method of Rubin et al. (6) as follows: each eye is graded separately

Table 21
Effects of SC-18862 in a 4-Hour Mouse Test

Treatment		Total Scores for 4 Mice*			
Dose mg/kg	Route of Admin.	Excitation†	Depression	Ataxia	Lethality
Controls	I.P.	21	0	0	0
5	"	21	0	0	0
20	"	21	0	3	0
40	"	20	0	3	0
80	"	21	0	1	0
320	"	21	0	1	0
Controls	S.C.	20	0	0	0
5	"	21	0	0	0
20	"	20	0	0	0
40	"	22	0	1	0
80	"	26	0	2	0
320	"	23	0	3	0

* Total possible scores for each index are as follows:
Excitation (100), Depression (100) and Ataxia (60).

† Control mice typically score 15-30 for excitation over the 4-hour period.

Table 22

Effects of SC-19192 in a 4-Hour Mouse Test

Treatment		Total Scores for 4 Mice*			
Dose mg/kg	Route of Admin.	Excitation†	Depression	Ataxia	Lethality
Controls	I.P.	15	0	0	0
5	"	19	0	2	0
20	"	21	0	5	0
40	"	18	4	6	0
80	"	20	0	1	0
320	"	21	0	2	0
Controls	S.C.	20	0	2	0
5	"	24	0	1	0
20	"	21	0	1	0
40	"	20	0	3	0
80	"	21	0	1	0
320	"	22	0	11	0

* Total possible scores for each index are as follows:
Excitation (100), Depression (100) and Ataxia (60).

† Control mice typically score 15-30 for excitation over the 4-hour period.

from 1 to 3; 1 (complete ptosis), 2 (partial ptosis) and 3 (no ptosis). According to this grading system, a group of 10 control mice showing complete ptosis in both eyes would be given a total score of 20. On the other hand, a group of 10 mice exhibiting no ptosis would be given the maximum total score of 60. Both SC-18862 and SC-19192 were tested at doses of 25 and 200 mg/kg intragastrically and the results of these tests are summarized in Table 23. SC-18862 was inactive at both 25 and 200 mg/kg. SC-19192 was inactive at 25 mg/kg and active in only one of three tests at 200 mg/kg. For comparative purposes, imipramine, nialamide and amphetamine were also included in this test. These drugs showed significant activity following oral doses of 25, 15 and 4.5 mg/kg respectively.

3. Effects on Hexobarbital Hypnosis in Mice

Groups of 16 male HAM/ICR mice weighing between 18 and 25 g were used for this procedure. Thirty minutes after intragastric administration saline (control), SC-18862 or SC-19192 mice were given intraperitoneal injections of hexobarbital, 100 mg/kg. Sleeptime was measured from the time each mouse lost its righting reflex until the animal righted itself spontaneously twice within a 15-second interval. A dose of test compound was rated active if it significantly altered sleeptime compared with concurrent saline treated controls (Student's *t* test, $P < 0.05$). Both SC-18862 and SC-19192 were tested at dose levels of 250, 500 and 1000 mg/kg intragastrically and the mean sleep times recorded at each dose level. As shown in Table 24, SC-18862 was inactive following doses of 250, 500 and 1000 mg/kg. SC-19192 was active only at the

Table 23
Antagonism of Reserpine-Induced Ptosis in Mice
(Test for Antidepressant Activity)

Treatment	Oral Dose mg/kg	Group Ptosis Score
Controls	—	20
SC-18862	25	20
	200	20
Controls	—	20
SC-19192	25	21
	200	30*
	200	20
	200	24
Controls	—	20
Imipramine	25	50*
	40	44*
Controls	—	20
Nialamide	5	20
	15	48*
	30	60*
Controls	—	20
Amphetamine	2	28
	4.5	44*
	9	44*

* Significantly different from concurrent control score by the Wilcoxon Rank Sum Test ($P < 0.05$).

Table 24
Effects of SC-18862 and SC-19192 on
Hexobarbital-Induced Sleeptime in Mice

Treatment	Oral Dose mg/kg	No. of Mice	Sleeptime (min.) mean \pm S.E.
Controls	--	16	40.6 \pm 3.2
SC-18862	250	16	49.2 \pm 3.0
	500	16	47.3 \pm 4.2
	1000	16	47.5 \pm 5.0
Controls	--	16	39.9 \pm 2.9
SC-19192	250	16	45.9 \pm 1.7
	500	16	43.4 \pm 3.4
	1000	16	48.9 \pm 2.6*

* Significantly different from controls ($P < 0.05$).

highest dose, 1000 mg/kg.

4. Effects on Motor Coordination in Mice

The method chosen for the estimation of effects on motor coordination was a variation of that described by Dunham and Miya (7). By this procedure, the endpoint for muscular coordination was the ability of male HAM/ICR mice weighing between 20 and 30 g to stay on a horizontal rotating (4.5 rpm) rod for 1 minute. A high percentage of normal (untreated) mice have no difficulty in staying on the rod for this period of time, whereas mice pretreated with tranquilizers, hypnotics, convulsants or muscle relaxants are not able to do this. Compounds are considered active in this test if a significant number of mice fall off the rod in comparison with the behavior of controls. Both SC-18862 and SC-19192 were administered intragastrically two and one-half hours prior to placing the mice on the rotating rod. As shown in Table 25, neither SC-18862 nor SC-19192 produced motor incoordination following oral doses of 50, 100 or 200 mg/kg. By way of comparison, diazepam, a potent tranquilizer and muscle relaxant, was significantly active in this test at a dose of 10 mg/kg.

5. Anticonvulsant Activity in Mice

Two tests for possible activity were carried out. The first test involved a measurement of the activity of SC-18862 or SC-19192 to abolish the hindlimb extensor component of the maximal electroshock seizure pattern in male HAM/ICR mice (20-30 g) by the method of Swinyard et al. (8). Two and one-half hours following oral adminis-

Table 25
 Test for Effects of SC-18862 and SC-19192
 on Motor Coordination in Mice

Treatment	Oral Dose mg/kg	No. of Mice Falling Off Rod
		No. Mice Tested
Controls	--	3/50
SC-18862	50	4/20
	100	1/10
	200	1/10
SC-19192	50	1/10
	100	2/10
	200	1/10
Diazepam	10	10/10*

* Significantly different from controls ($P < 0.05$).

istration of the test compounds, each mouse is challenged with a current of 50 milliamperes delivered via corneal electrodes. This current is sufficient to induce maximal electroshock seizures in essentially 100 per cent of control animals. A dose of test compound is rated active if a significant number of mice tested are protected against the hindlimb extensor component of the seizure. The results of these tests are summarized in Table 26. As shown, both SC-18862 and SC-19192 were devoid of anticonvulsant activity at oral doses as high as 200 mg/kg. By way of comparison, diphenylhydantoin, a known anticonvulsant agent, was significantly active following an oral dose of 15 mg/kg.

In the second test, groups of mice were given oral doses of either SC-18862 or SC-19192 and one and a half hours later 35 mg/kg of Metrazol was administered intravenously. This dose of Metrazol will produce maximal clonic convulsions in control mice. Abolition of clonic seizures in a significant number of mice tested was used as an indication of activity. As shown in Table 27, both SC-18862 and SC-19192 were inactive in this test at oral doses as high as 200 mg/kg. On the other hand, clonic seizures are well controlled by two known anticonvulsant agents, trimethadione and diazepam, with calculated ED_{50} s of 96 and 0.8 mg/kg respectively.

6. Analgesic Activity in Mice

Two tests for possible analgesic activity of SC-18862 and SC-19192 were carried out. The first test, a modification of the method described by Eddy and Leimbach (9) involved the ability of a compound to increase reaction time to a thermal stimulus. In this test, groups of

Table 26
Effects of SC-18862 and SC-19192 on
Maximal Electroshock Seizures in Mice

Treatment ^a	Oral Dose mg/kg	No. Mice Protected
		No. Mice Tested
Controls	--	0/50
SC-18862	50	0/20
	100	0/10
	200	0/10
SC-19192	50	1/10
	100	0/10
	200	0/10
Diphenylhydantoin	15	7/10*

^a

Treatment was given 2 1/2 hours prior to maximal electroshock.

* Significantly different from controls ($P < 0.05$).

Table 27
Effects of SC-18862 and SC-19192 on
Metrazol-Induced Clonic Seizures in Mice

Treatment	Oral Dose mg/kg	<u>No. Mice Protected</u> <u>No. Mice Tested</u>	ED ₅₀ mg/kg
Controls	--	0/10	-
SC-18862	100	1/10	
	200	0/10	>200
SC-19192	100	0/10	
	200	0/10	>200
Trimethadione	50	2/20	
	100	13/20	
	200	16/20	96
Diazepam	0.5	2/10	
	1.0	6/10	
	2.0	10/10	0.8

10 male HAM/ICR mice supplied by Charles River weighing between 18 and 25 g were placed individually in a restraining cylinder on a hot plate with the temperature controlled at $55 \pm 0.3^{\circ}\text{C}$. The reaction time of each mouse to lick a foot or jump was measured at 60, 40 and 20 minutes before and 30, 60, 90 and 120 minutes after administration of the test compound. The "normal" reaction time was measured as the median of the three pretreatment times. A positive response consisted of a reaction time greater than twice the normal time at any of the post-treatment trials. A dose of compound was considered active if 50 per cent or more of the treated group responded positively. SC-18862 and SC-19192 were tested at 50 and 100 mg/kg intragastrically and the test was repeated at each dose level. The results of these tests are summarized in Table 28. As shown, both SC-18862 and SC-19192 were inactive in this test.

A second analgesic test was carried out using a modification of Haffner's (10) method as described by Bianchi and Franceschini (11). Adult male Charles River mice weighing between 18 and 25 gm are used in this test. A pressure-standardized artery clip is placed approximately one inch from the base of the tail and only the mice that respond to the clip by turning or biting at the clip within 15 seconds are used for the test. The response times for these mice are recorded and groups of 10 mice each are used to test each dose level of each test compound. The response time to the clip is determined at 30 minute intervals up to two hours following intragastric administration of the test compounds. An analgesic effect is considered positive in each mouse if the response time at any of the four post-treatment intervals is twice the pretreatment

Table 28
 Test for Analgesic Activity in Mice
 Using the Hot Plate Test

Treatment	Dose, i.g. mg/kg	No. Mice Showing Positive Response
		No. Mice Tested
Controls	--	0/10
SC-18862	50	3/10
	100	1/10
Controls	--	1/10
SC-18862	50	0/10
	100	1/10
Controls	--	0/10
SC-19192	50	2/10
	100	0/10
SC-19192	50	2/10
	100	2/10

response time. The results of these tests are summarized in Table 29. As shown, both SC-18862 and SC-19192 were devoid of analgesic activity following oral doses of 50 and 100 mg/kg.

7. Central Anticholinergic Activity in Mice

Antagonism of tremorine-induced incoordination was used to test for central anticholinergic activity of SC-18862 and SC-19192. In this test, male HAM/ICR mice supplied by Charles River are challenged with an intraperitoneal injection of 20 mg/kg of tremorine dihydrochloride 20 minutes following oral or intraperitoneal administration of the test compounds. Ten minutes after tremorine injection, the mice are placed on a rotating rod. Mice protected by an active compound will stay on the rod for at least two minutes. Unprotected mice (inactive compounds) are unable to stay on the rod for this period of time. A dose of test compound is considered active if a significant number of the treated mice stay on the rod compared to controls. As shown in Table 30, both SC-18862 and SC-19192 were inactive following oral doses of 200 mg/kg. SC-18862 was also tested intraperitoneally at a dose of 20 mg/kg and was found to be inactive. On the other hand, trihexyphenidyl, a drug known to possess central anticholinergic activity, was significantly active intraperitoneally at a dose of 10 mg/kg.

8. Effects on Behavior in Rats

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Various doses of SC-18862, SC-19192 and L-phenylalanine were

Table 29
Test for Analgesic Activity in Mice
Using the Tail Clip Method

Treatment	Dose, i.g. mg/kg	<u>No. Mice Showing Positive Response</u>
		<u>No. Mice Tested</u>
Controls	--	0/10
SC-18862	50	0/10
	100	0/10
Controls	--	0/10
SC-19192	50	0/10
	100	0/10

Table 30
 Test for Central Anticholinergic Activity
 (Tremorine Antagonism in Mice)

Treatment ^a	Dose mg/kg	Route of Admin.	No. Mice Protected
			No. Mice Tested
Controls	--	--	1/40
SC-18862	20	I.P.	0/6
	200	I.G.	0/16
SC-19192	20	I.G.	0/6
	200	I.G.	0/6
Trihexyphenidyl	1	I.P.	2/10
	3	I.P.	4/10
	10	I.P.	6/10*
	30	I.P.	9/10*

^a Compounds were administered 20 minutes prior to intraperitoneal injection of 20 mg/kg of tremorine dihydrochloride. The mice were then tested for motor coordination 10 minutes after the tremorine injection.

* Significantly different from controls ($P < 0.05$).

administered to rats and the effects on their subsequent behavior were evaluated. Naive male Fischer rats approximately 90 days of age were treated intragastrically (IG) with either saline or 50, 100 or 200 mg/kg of one of the three compounds. Thirty minutes following treatment each rat was placed in an apparatus consisting of a shuttle box divided into two compartments and enclosed in a sound attenuating chamber. The floor of the shuttle box was an electrifiable grid. A five second conditioned stimulus, consisting of a tone and a light, preceded a 0.2 ma. footshock delivered to the grid floor of the cage. The shock was automatically terminated after 30 seconds if the rat failed to respond. A shuttle response during the conditioned stimulus prevented the onset of the shock and was scored as an avoidance response. A shuttle response during the shock period terminated the conditioned stimulus and the shock and was scored as an escape response. If no escape response was made, an escape failure was scored. Each conditioned stimulus presentation was separated by a 15 second interval and a response during this time resulted in the onset of the shock and the conditioned stimulus until the rat returned to the other side. Each rat was presented with 100 trials (i.e., 100 conditioned stimulus presentations) which takes approximately thirty minutes. Twelve rats per dose were tested and each dose group was compared to the control group run concurrently by means of Student's t test ($P \leq .05$, two tailed).

The data are summarized in Tables 31-33 for each of the compounds. Neither SC-18862 nor L-phenylalanine had any significant effect at any dose tested. SC-19192 had a significant effect on the trial number

Table 31

Effects of Various I.G. Doses of SC-18862 on Several Measures of Performance in Rats
(Data are expressed as the mean \pm standard error)

	Dose mg/kg		
	Saline (control)	50	100
Total Avoidance Responses	15.08 \pm 1.32	18.08 \pm 2.11	22.08 \pm 3.45
Avoidance Responses Trials 1-50	3.17 \pm .49	3.42 \pm .60	4.50 \pm .65
Avoidance Responses Trials 51-100	11.92 \pm 1.51	14.67 \pm 1.78	17.58 \pm 3.22
First Avoidance Response	32.50 \pm 5.24	23.58 \pm 6.44	21.25 \pm 4.86
Intertrial Interval Responses	26.00 \pm 4.26	23.42 \pm 3.19	24.50 \pm 3.21
Escape Latency (seconds)	.62 \pm .08	.56 \pm .07	.65 \pm .06

⁺ Data for these measures are based on 8 rats rather than 12 since part of the data was lost due to a computer failure.

Table 32

Effects of Various I.G. Doses of SC-19192 on Several
Measures of Performance in Rats
(Data are expressed as the mean \pm standard error)

	Dose (mg/kg)		
	Saline (control)	50	100
Total Avoidance Responses	18.50 \pm 2.47	19.50 \pm 2.71	19.17 \pm 2.64
Avoidance Responses Trials 1-50	4.00 \pm .70	5.58 \pm .79	4.92 \pm .73
Avoidance Responses Trials 51-100	14.50 \pm 2.11	13.92 \pm 2.05	14.25 \pm 2.28
First Avoidance Response	31.25 \pm 4.68	12.67* \pm 2.40	21.83 \pm 4.70
Intertrial Interval Responses	24.67 \pm 2.89	27.25 \pm 3.47	30.72 \pm 8.09
Escape Latency (seconds)	.42 \pm .04	.38 \pm .03	.42 \pm .04

* $P \leq .05$, two tailed t test.

*

*

Table 33

Effects of Various I.G. Doses of L-Phenylalanine on
Several Measures of Performance in Rats
(Data are expressed as the mean \pm standard error)

	Doses (mg/kg)		
	Saline (Control)	50	100
Total Avoidance Responses	22.50 \pm 2.73	19.83 \pm 3.44	20.83 \pm 3.44
Avoidance Responses Trials 1-50	4.50 \pm .72	4.67 \pm .92	5.00 \pm .91
Avoidance Responses Trials 51-100	18.00 \pm 2.28	15.17 \pm 3.23	15.08 \pm 2.12
First Avoidance Response	24.25 \pm 4.67	21.82 \pm 4.22	20.83 \pm 4.67
Intertrial Interval Responses	33.50 \pm 4.01	31.50 \pm 5.82	27.75 \pm 5.00
Escape Latency (seconds)	.59 \pm .05	.59 \pm .05	.52 \pm .04

21.25 \pm 3.354.00 \pm .6017.25 \pm 3.0220.58 \pm 4.1930.25 \pm 4.66.59 \pm .05

of the first avoidance response at the 50 and 200 mg/kg dose. However, since this effect was not dose related and was not accompanied by a significant increase in intertrial interval responses it may reflect experimental error, biological variability or statistical probability. [Compounds with stimulant activity often significantly decrease the first avoidance trial number but always cause a concomitant increase in intertrial interval responses.]

In summary, it can be concluded that SC-18862, SC-19192 and L-phenylalanine exert no significant effect on conditioned avoidance response performance in naive rats following a single oral administration of 50, 100 or 200 mg/kg.

D. Miscellaneous Pharmacological Actions

1. Diuretic Activity in Rats

The possible diuretic effects of SC-18862 and SC-19192 were assessed in intact Sprague-Dawley male rats, 188-228 gm, by Van Arman's (12) modification of the method described by Lipschitz et al. (13). The animals were maintained on a normal laboratory diet with water ad libitum. Food was withdrawn 18 hours before and water removed during the five-hour test. Both compounds were administered orally at a high oral dosage of 100 mg/kg in 0.9% NaCl (25 ml/kg) and the effects on urine volume and electrolyte excretion observed for five hours. Six groups of four rats per treatment were placed in metabolism cages with a complete urine sample collected following induced voiding via gentle bladder palpation. Table 34 summarizes the mean responses, together with reference data for untreated controls. Both SC-18862 and SC-19192

Table 34
Test for Diuretic Activity in Rats

Treatment	N	Mean Urinary Response/5 hr ^{a,b}		
		Urine ml	μ Eq	
			Na	K
Control	6	9.8 \pm 1.4	1.97 \pm 0.25	0.48 \pm 0.05
SC-18862	6	9.1 \pm 0.6	1.96 \pm 0.12	0.44 \pm 0.08
SC-19192	6	8.2 \pm 0.8	1.84 \pm 0.24	0.47 \pm 0.07

^a 6 groups of rats (4 animals per group) were dosed orally with 25 ml/kg of isotonic saline containing either SC-18862 or SC-19192 at a dosage of 100 mg/kg. Urine collected for 5 hrs.

^b Mean \pm 95% fiducial limits for urine volume and Na and K excretion for control bracketed the responses for both test compounds.

failed to exhibit a diuretic effect at the 100 mg/kg dosage employed. All urinary parameters fell within the fiducial limits of control responses. By way of comparison, hydrochlorothiazide, a known diuretic agent has been reported by Hofmann (14) to induce a significant increase in urine volume and Na and K excretion in this same test at a dosage of 0.1 mg/kg.

2. Effects on Blood Glucose in Rats

SC-18862 and SC-19192 were tested for their effects on blood glucose in adult, fasted Sprague-Dawley rats supplied by Charles River. Briefly, male Charles River rats weighing 180-230 g were fasted for twenty-four hours. Blood samples were then obtained from the tail veins and analyzed for serum glucose concentration by the method of Asrow (15). One hour later SC-18862 or SC-19192 was administered intragastrically. Serum samples were again obtained two and four hours after injection and analyzed for glucose content. As shown in Table 35, neither SC-18862 nor SC-19192 had any significant effect on blood glucose following oral doses of 100 mg/kg. On the other hand, chlorpropamide, a known hypoglycemic agent, markedly reduced serum glucose following an oral dose of 100 mg/kg.

3. Effects on Body Weight Gain and Blood Cholesterol in Hypercholesterolemic Rats

Male Charles River rats (200-250 g) made hypercholesterolemic with propylthiouracil as described by Ranney and Saunders (16) were used to determine the effects of SC-18862 and SC-19192 on body weight gain

Table 35
Effects of SC-18862 and SC-19192 on Blood
Serum Glucose in Adult Rats

Treatment	Oral Dose mg/kg	No. of Rats	Mean mg% Glucose \pm S.E.		
			Control	2 hrs	4 hrs
SC-18862	100	4	78 \pm 2.7	83 \pm 4.6	85 \pm 4.0
SC-19192	100	4	75 \pm 1.9	81 \pm 2.7	78 \pm 5.5
Chlorpropamide	100	4	66 \pm 2.1	35 \pm 1.6*	38 \pm 1.9*

* Significantly different from control mean (P < 0.05)

and blood cholesterol. Groups of eight rats were treated intragastrically daily for nine days with solutions of the compounds in 30% propylene glycol. The control group of rats were given the vehicle only. All animals received propylthiouracil, 0.02%, in their drinking water. On the tenth day each rat was anesthetized with ether and blood samples were withdrawn from the abdominal aorta. Serum samples were then analyzed for total cholesterol by the method of Black et al. (17). Body weights were taken on the first and tenth day of the test to determine if the compounds had any effects on body weight gain. The results of these tests are summarized in Table 36. As shown, neither SC-18862 nor SC-19192 at oral doses of 5, 10, 30 and 200 mg/kg had any significant effects on body weight gain or blood serum cholesterol.

4. Antiacetylcholine Activity In Vitro

A test for anticholinergic activity was carried out using the method of Miller et al. (18) on segments of rabbit ileum cleansed of adipose tissue and suspended in Tyrode's solution. Muscle movement was measured using isotonic myograph transducers and recorded on a physiograph. In testing, maximum muscle contraction was induced by using a standard concentration of acetylcholine (5 $\mu\text{g/ml}$) in the muscle bath. SC-18862, SC-19192, or the standard, atropine sulfate, was then added in increasing amounts until concentrations were found that relaxed the amplitude of the contraction less than and greater than 50 per cent. Each concentration was tested over a 7-minute interval with at least 8-minute intervals between tests to provide for rinsing of

Table 36

Effects of SC-18862 and SC-19192 on Body Weight Gain
and Blood Cholesterol in Propylthiouracil-Induced
Hypercholesterolemic Rats (10-day test)

Treatment	Oral Dose mg/kg/day	No. of Rats	Weight Gain mean \pm S.E. gm	Serum Cholesterol mean \pm S.E. mg%
Controls	--	8	46 \pm 2.7	56 \pm 2.6
SC-18862	5	8	47 \pm 4.2	51 \pm 2.4
	10	8	48 \pm 3.2	61 \pm 3.5
	30	8	54 \pm 2.2	58 \pm 1.5
	200	8	36 \pm 5.7	55 \pm 2.2
SC-19192	5	8	34 \pm 8.6	60 \pm 3.3
	10	8	51 \pm 2.4	56 \pm 2.1
	30	8	51 \pm 1.8	60 \pm 1.8
	200	7	49 \pm 2.4	61 \pm 2.3

the muscle strip and replacing the bath solution. The data resulting from these tests are summarized in Table 37. As shown, concentrations of SC-18862 or SC-19192 one thousand times those used for atropine sulfate failed to reduce the amplitude of contraction by 50 per cent indicating that the anticholinergic activity, if present at all, is less than 0.001 that of atropine.

5. Antihistamine Activity In Vitro

Tests for antihistamine activity were carried out using segments of guinea pig ileum suspended in Tyrode's solution. Muscle movement was recorded using myograph transducers and a physiograph. In testing, maximum muscle contraction was induced by a standard concentration of histamine diphosphate (50 $\mu\text{g/ml}$) in the muscle bath. A concentration of the standard antihistamine agent, diphenhydramine hydrobromide, was then added to the bath to induce relaxation. Each concentration used was tested over a 7-minute interval with at least 8-minute intervals allowed between tests to provide for rinsing of the tissue and replacing the bath solution. This entire procedure was repeated at increasing concentrations of diphenhydramine until two concentrations produced less than and greater than 50 per cent relaxation. The same procedure was repeated using SC-18862 and SC-19192. As shown in Table 38, diphenhydramine at pD concentrations of 6.3 to 7.0 produced relaxations greater than 50 per cent. Both SC-18862 and SC-19192 at a pD concentration of 4.6 failed to relax the tissue by 50 per cent indicating that both compounds are essentially devoid of antihistaminic activity.

Table 37
Inhibition of Acetylcholine-Induced
Spasm of Isolated Rabbit Ileum

Treatment	No. of Muscle Strips	Concentration pD*	Relaxation %	Relative Potency vs Atropine
Atropine SO ₄	2	8.6	13	
		7.6	78	
SC-18862		4.6	15	< 0.001
Atropine SO ₄	2	8.6	28	
		7.6	93	
SC-19192		4.6	3	< 0.001

*pD = negative log of the reciprocal of the dilution ratio expressed
in gm/ml.

Table 38

In Vitro Test for Antihistamine Activity
Using Guinea Pig Ileum

Treatment	No. of Strips	Concentration pD*	Relaxation %	Relative Potency vs Diphenhydramine
Diphenhydramine	2	6.5	39	
		6.3	56	
SC-18862		4.6	15	< 0.02
Diphenhydramine	2	7.8	17	
		7.0	86	
SC-18862		4.6	16	< 0.004
Diphenhydramine	2	7.8	15	
		7.0	62	
SC-19192		5.4	10	
		4.6	23	< 0.004
Diphenhydramine	2	6.6	15	
		6.4	58	
SC-19192		5.4	3	
		4.6	15	0.010

* pD = log of the reciprocal of the dilution ratio expressed in g/ml.

6. Autonomic Ganglionic Blockade

SC-18862 and SC-19192 were tested for their effects on the superior cervical ganglia of cats using a modification of the method of Acheson and Pereira (19). Each cat was anesthetized with sodium pentobarbital and the following operative procedures were carried out: (a) the right superior sympathetic nerve was exteriorized and sectioned caudad to the superior cervical ganglion, (b) the left common carotid artery was cannulated to monitor blood pressure, (c) a femoral vein was cannulated for injection of compounds. The rostral stump of the cervical sympathetic nerve was stimulated electrically and the sustained contraction of the ipsilateral nictitating membrane was recorded on a physiograph. SC-18862 and SC-19192 dissolved in saline were tested at a single intravenous dose of 6.4 mg/kg. Tetraethylammonium bromide was also tested in each cat to verify that each preparation would respond to a known ganglionic blocking agent. As shown in Table 39, both SC-18862 and SC-19192 were devoid of sympathetic ganglionic blocking activity following an intravenous dose of 6.4 mg/kg.

7. Effect of Dietary Administration on Serum Levels of Glucose, Insulin, Triglycerides, Free Fatty Acids and Cholesterol in Rats

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The following is a description of the methods and a brief summary of the results. A complete report of this study may be found in the appendix.

Table 39
Test for Sympathetic Ganglionic Blockade in Cats

Cat No.	Treatment		Nictitating Membrane Relaxation %
	Compound	Dose mg/kg, i.v.	
1	TEA bromide (a)	0.8	46
		1.6	87
	SC-18862	6.4	0
2	TEA bromide	1.6	48
		3.2	75
	SC-18862	6.4	0
3	TEA bromide	0.4	9
		0.8	55
	SC-19192	6.4	0
4	TEA bromide	0.4	32
		0.8	53
	SC-19192	6.4	0

(a)

TEA bromide = tetraethylammonium bromide.

Male and female Charles River, DC, rats weighing between 150 and 200 g were placed in metabolism cages and fed ground stock diet (Rockland Rat Diet) containing either 0.2% or 2% of SC-18862 or SC-19192. These two percentages of compound in the diet were estimated to yield an average daily consumption of 0.2 and 2 gm of compound per kg of body weight. Ten rats of each sex were used at the two treatment levels for both compounds with an equal number of corresponding control rats fed only the ground stock diet. The paired-feeding technique was used in which a rat of the same sex and similar weight was given the amount of ground stock diet plus one additional gram that was eaten by its paired-treated rat. Body weight, food and water consumption were measured daily. After seven consecutive days of treatment, the rats in the fed state were sacrificed by decapitation and the blood was allowed to drain into beakers. After clotting occurred, the serum was separated by centrifugation. Standard laboratory techniques were used to measure the serum levels of glucose, insulin, free fatty acids, triglycerides and cholesterol.

Results - SC-18862

For the treated rats the mean daily consumption of SC-18862 in grams per kilogram of body weight were as follows: females low dose, .152; females high dose, 1.48; males low dose, .200; males high dose, 2.01. These treatment levels when ingested by the rats for one week did not significantly ($P > 0.05$) affect the serum levels of glucose, insulin, free fatty acids, triglycerides or cholesterol when compared to the corresponding values for control rats.

Results - SC-19192

For the treated rats the mean daily consumption of SC-19192 in grams per kilogram of body weight were as follows: females low dose, .153; females high dose, 1.57; males low dose, .197; males high dose, 1.88. These treatment levels when ingested by the rats for one week did not significantly ($P > 0.05$) affect the serum levels of glucose, insulin, free fatty acids, triglycerides or cholesterol when compared to the corresponding values for control rats.

E. Summary

SC-18862, as well as its degradation product, a diketopiperazine (SC-19192), were subjected to a wide variety of pharmacological tests in order to delineate any possible adverse effects on the gastrointestinal, cardiovascular or the central nervous systems. In addition, several miscellaneous tests were also carried out.

Tests related to the gastrointestinal system showed that neither SC-18862 nor SC-19192 inhibited appetite in rats following a high oral dose of 200 mg/kg. No significant effects on gastric juice volume, acidity or proteolytic activity could be demonstrated in rats following oral doses of 50 mg/rat (approximately 250 mg/kg). Likewise, neither compound potentiated or inhibited ulceration induced in rats by pyloric ligation. In vitro tests also showed that SC-18862 and SC-19192 did not inhibit the proteolytic activity of pepsin or the lipolytic activity of pancreatic lipase.

Tests for effects of SC-18862 and SC-19192 on the cardiovascular system showed that (a) intravenous administration to anesthetized dogs had no significant effects on blood pressure at doses of 0.1 or 1.0 mg/kg and 5.0 mg/kg of SC-18862 induced only a slight transient fall in blood pressure, (b) oral administration of 100 or 200 mg/kg did not significantly alter blood pressure or heart rate in unanesthetized dogs, (c) intravenous administration of 10 mg/kg did not alter the pressor response to angiotensin in anesthetized rats, (d) no effects could be demonstrated on aconitine-induced arrhythmia in the isolated rabbit heart indicating the absence of quinidine-like activity, and (e) neither compound possessed

in vitro blood anticoagulant activity.

Several tests were carried out to determine whether or not SC-18862 or SC-19192 might have any effects on the central nervous system as follows: (a) a test for general effects in mice was conducted by observing the animals for several hours following subcutaneous or intraperitoneal administration of 5, 20, 40, 80 or 320 mg/kg. Neither compound caused excitation or depression at any of these doses. It was concluded that the very slight ataxia observed was not biologically significant because no dose-response relationship existed and a more objective measurement for effects on motor coordination was negative for both SC-18862 and SC-19192 following oral doses of 50, 100 or 200 mg/kg, (b) no antidepressant activity could be demonstrated in mice at oral doses as high as 200 mg/kg, (c) SC-18862 did not potentiate hexobarbital sleeptime in mice at oral doses of 250, 500 or 1000 mg/kg. SC-19192 slightly potentiated sleeptime only at the highest dose, 1000 mg/kg, (d) two tests for anticonvulsant activity in mice showed both compounds to be inactive following oral doses of 50, 100 or 200 mg/kg, (e) two tests for analgesic activity in mice showed both compounds to be inactive following oral doses of 50 or 100 mg/kg, (f) no central anticholinergic activity could be demonstrated following an oral dose of 200 mg/kg in mice, and (g) no significant effect on conditioned avoidance response performance of naive rats could be demonstrated following oral doses of 50, 100 or 200 mg/kg. L-phenylalanine was also subjected to this behavioral test at the same dosage levels and was found to be inactive.

Other pharmacological screening tests carried out showed that SC-18862 and SC-19192 were (a) inactive as diuretic agents in rats at oral doses of 100 mg/kg, (b) did not affect blood glucose levels in rats following oral doses of 100 mg/kg, (c) did not affect blood serum cholesterol or body weight gain in hypercholesterolemic rats following daily oral administration of 5, 10, 30 or 200 mg/kg for nine days, (d) did not possess antiacetylcholine or antihistamine activity in vitro, and (e) did not possess sympathetic ganglionic blocking activity in anesthetized cats following intravenous doses of 6.4 mg/kg.

In addition to the above tests, a study was carried out to determine if dietary administration of SC-18862 or SC-19192 at either a low (0.2%) or a high (2%) concentration to male and female rats for a period of one week had any effects on blood serum levels of glucose, insulin, triglycerides, free fatty acids or cholesterol. The actual mean daily consumption of SC-18862 was 0.2 and 2.01 gm/kg for male rats and 0.152 and 1.48 gm/kg for female rats. None of these dosage levels had any significant effects on the blood serum parameters measured. For SC-19192, the mean daily consumption was 0.197 and 1.88 gm/kg for male rats and 0.153 and 1.57 gm/kg for female rats. None of these dosage levels of SC-19192 had any significant effects on the blood serum parameters measured.

Based on the results of the wide variety of pharmacological tests summarized in this report, it can be concluded that neither SC-18862 nor its degradation product, SC-19192, would be expected to possess any pharmacological activity when used orally as a sweetening agent in man.

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G. Appendix

Effect of Dietary Administration of SC-18862 and SC-19192 on Serum Levels of Glucose, Insulin, Triglycerides, Free Fatty Acids and Cholesterol in Rats

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Insulin levels in the blood are known to be increased in man and animals by the ingestion of high meat meals (1, 2). Amino acids have been demonstrated to increase the level of circulating insulin whether given orally, intravenously or in the form of a protein meal (1, 2, 3, 4). Insulin release by amino acids has been suggested to occur by both a direct stimulant effect on the pancreatic stores of insulin (β -cells) and by an indirect mechanism through increased circulating levels of secretin and pancreozymin (5). The potency order of amino acids with respect to their insulin releasing capacity in man are as follows: arginine > lysine > leucine > phenylalanine > tryptophane > valine > methionine > histidine (6). In the rat this order is slightly different as indicated by the following potency correlation: leucine > arginine > phenylalanine > lysine > tryptophane > histidine > valine > methionine (6).

SC-18862 is the methyl ester of aspartyl phenylalanine. After oral ingestion, SC-18862 is hydrolyzed to phenylalanine and aspartic acid by the digestive process. Phenylalanine was indicated by Malaisse and Malaisse-Lagae (6) to be an effective enhancer of insulin release from the rat pancreas when glucose is also present. Literature reports on the effect of aspartic acid on circulating insulin levels were not

found. If the ingestion of SC-18862 in elevated amounts were to result in high blood levels of phenylalanine, then an effect on the level of circulating insulin levels might be anticipated. Increased blood insulin levels would be expected to alter the blood levels of glucose, free fatty acids, triglycerides and cholesterol. This study was initiated to determine if the feeding of SC-18862 in high levels would alter the blood levels of the mentioned metabolic parameters in rats.

SC-19192 is the diketopiperazine derivative of SC-18862. SC-19192 occurs in low concentrations in the preparation of SC-18862 used in this study and was therefore evaluated for effects on metabolic parameters under the same protocol used for SC-18862.

Methods:

Male and female Charles River rats weighing between 150 and 200 grams were placed in metabolism cages and fed ground stock diet (Rockland Rat Diet) containing either 0.2% or 2% of SC-18862 or SC-19192. These two percentages of compound in the diet were estimated to yield an average daily consumption of 0.2 and 2 grams of compound per kilogram of body weight. Ten rats of each sex were used at the two treatment levels for both compounds with an equal number of corresponding control rats fed only the ground stock diet. The paired-feeding technique was used in which a rat of the same sex and similar weight was given the amount of ground stock diet plus one additional gram that was eaten by its paired-treated rat. Body weight, food and water consumption were measured daily. After seven consecutive days of treatment, the rats in the fed state were sacrificed by decapitation

and the blood was allowed to drain into beakers. After clotting occurred the serum was separated by centrifugation. The serum was analyzed for insulin (Phadebas Radioimmuno/assay Kit, using rat insulin as standard), free fatty acids (7), triglycerides (8) and cholesterol (9) in the bioanalytical laboratory utilizing standard techniques. Serum glucose values were determined by using a Glucose Analyzer (Beckman) maintained in the pharmacology department.

Results:

The mean daily consumption of SC-18862 was similar to the anticipated amounts with the male rats but less than anticipated with the female rats as indicated in Table 1. This is directly related to the lower food consumption of the females compared to the males as recorded in Table 2. The females receiving the lower level of SC-18862 ate significantly more food than their pair-fed controls. This result is not assumed to be related to the presence of SC-18862 in the diet but a result of the pair-feeding technique which tends to restrict the feeding of the controls. The pair-feeding technique is not useful when the treated rats consume a normal amount of diet but is essential when treatment reduces the amount of diet consumed.

The results from one of the control female rats (paired to a rat in the high SC-18862 diet level group) was omitted from Table 2 and the remaining tables because the animal lost weight during the one week study. The food consumption data on this rat was normal therefore the weight loss was felt to indicate an unhealthy state. All the values of the metabolic parameters from this rat were within the range of the

reported control group.

Table 3 indicates that three of the SC-18862 treated groups, females low and high dose and males low dose, gained significantly more weight over the one week interval than their pair-fed controls. This effect again was not felt to be related to the presence of SC-18862 in the diet but a result of the restriction of the control rats normal feeding habits by the paired-feeding technique. Water consumption as recorded in Table 4 did not significantly differ between the treated and control groups.

Serum levels of cholesterol, triglyceride and free fatty acids indicated in Table 5 were comparable between the SC-18862 treated groups and their respective pair-fed control groups. Serum levels of glucose and insulin indicated in Table 6 were similar between the SC-18862 treated groups and their respective pair-fed controls.

A similar protocol was utilized in the evaluation of the effect of SC-19192 on the same metabolic parameters. The mean daily consumption of SC-19192 in grams per kilogram of body weight for the male rats was similar to the anticipated value (Table 7). The female groups compound consumption was lower than anticipated for reasons expressed in the results of the SC-18862 section. Three of the female rats (two low dose controls and one low dose treated) lost weight over the one week study. The data from these animals were omitted from the results. The data from two additional female rats (one low dose treated and one low dose control) were omitted from the results since these animals lacked water (accidental omission of water bottle from cage) on the

last day of the study. Lack of water resulted in reduced food ingestion in these two animals which in turn would be expected to alter the levels of metabolic parameters measured.

Table 8 indicates that the SC-19192 treated groups and their respective control groups had similar mean weekly food consumption values with the exception of the female group on the higher level of SC-19192 which consumed significantly more than their pair-fed controls. This was again assumed to be a result of the pair-feeding technique and not an indication that the rats preferentially ingested more of the diet containing SC-19192 than they would have if the diet lacked SC-19192.

Male rats on the lower level of SC-19192 had a greater weight gain than their respective pair-fed controls (Table 9) whereas the other groups demonstrated no significant difference in mean weight gain over the one week study. Water consumption as shown in Table 10 was also greater in the two groups given the higher levels of SC-19192 in their diet.

Serum levels of cholesterol, triglyceride and free fatty acid were similar between the SC-19192 treated groups and their respective control group (Table 11). Table 12 indicates that serum insulin and glucose values were comparable between the SC-19192 treated and their respective control group.

To determine whether food intake of the treated and control groups would be similar if both groups were allowed to feed ad libitum, one additional feeding study was designed. The per cent of SC-18862

in the ground stock diet was increased to 2.7% which was calculated to yield an average daily compound consumption of 2 grams per kilogram of body weight at the normal food consumption rate of female rats. Table 13 indicates that the anticipated ingestion levels of SC-18862 was achieved. Tables 14 through 18 indicate that this level of SC-18862 did not alter the food or water consumption, increase in body weight or level of metabolic parameters of the treated group when compared to the control group.

Summary:

SC-18862 and SC-19192 were added to the diets of male and female rats at levels anticipated to achieve daily consumption of 0.2 or 2.0 grams of compound per kilogram of body weight. Increased diet consumption was noted in one of the SC-18862 treated and one of the SC-19192 treated groups. This was assumed to be the result of the pair-feeding technique. When the control group was allowed to feed ad libitum as in the last section of this study, the addition of SC-18862 to the diet did not influence the amount of diet consumed by the rats.

The increased weight gain of several SC-18862 groups and one SC-19192 group compared to their respective control was assumed to be related to the increased diet consumed.

The addition of SC-18862 or SC-19192 to the diets for an interval of one week did not alter the serum levels of insulin, free fatty acids, triglycerides, glucose or cholesterol. Thus this study suggests that the blood levels of the natural amino acids, phenylalanine

and aspartic acid, which resulted from these treatment levels, did not exhibit an abnormal effect on intermediary metabolism.

References

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Table 1.
Daily Amount of SC-18862 Consumed

No. of Rats	Sex	Anticipated SC-18862 Consumption	Grams of SC-18862 Consumed per Kilogram of Body Weight Each Day (Mean \pm S.E.)							Mean (Gm/Kg) Daily Consumption (Mean \pm S.E.)
			1	2	3	4	5	6	7	
10	Female	0.2 Gm/Kg	.161 \pm .009	.148 \pm .010	.149 \pm .010	.164 \pm .008	.146 \pm .007	.146 \pm .007	.147 \pm .008	.152 \pm .003
10	Male	0.2 Gm/Kg	.194 \pm .007	.214 \pm .008	.187 \pm .018	.205 \pm .006	.182 \pm .013	.203 \pm .004	.216 \pm .029	.200 \pm .005
10	Female	2 Gm/Kg	1.24 \pm .14	1.67 \pm .23	1.60 \pm .08	1.68 \pm .13	1.48 \pm .07	1.29 \pm .12	1.45 \pm .06	1.48 \pm .07
10	Male	2 Gm/Kg	2.06 \pm .22	2.10 \pm .09	2.21 \pm .26	1.97 \pm .10	2.14 \pm .29	2.03 \pm .09	2.01 \pm .08	2.07 \pm .03

Table 2.
Food Consumption

No. of Rats	Sex	SC-18862 (Gm/Kg) Consumed (Mean±S.E.)	1	2	3	4	5	6	7	Weekly Food Consumption (Gm) (Mean±S.E.)
10	Female	.152±.003	16.0±1.0	15.0±1.0	15.4±1.2	17.3±1.1	15.5±.9	15.6±.9	16.1±1.1	111±4*
10	Female	Pair-Fed Control	12.3±.5	13.4±.9	12.6±1.0	14.3±.7	14.1±.7	14.3±.7	14.1±1.1	95±3
10	Male	.200±.005	15.8±.7	18.2±.7	16.6±1.6	19.0±.6	17.6±1.3	20.3±.5	22.4±2.8	130±3
10	Male	Pair-Fed Control	14.8±.5	17.1±.7	15.8±1.5	18.1±.7	17.0±1.1	19.8±.5	20.4±1.4	122±4
10	Female	1.48 ± .07	12.1±1.5	16.3±2.2	15.9±.9	17.0±1.2	15.2±.7	13.4±1.3	15.2±.6	105±4
9†	Female	Pair-Fed Control	11.0±1.1	14.1±1.0	14.2±.9	15.2±.4	15.0±.6	13.3±1.4	15.2±.5	98±3
10	Male	2.07 ± .03	16.5±1.8	17.3±.9	18.9±2.1	17.7±1.2	20.3±3.2	19.8±1.3	20.0±.9	130±8
10	Male	Pair-Fed Control	13.6±1.1	16.6±.7	16.2±1.7	16.7±1.0	16.5±1.5	19.3±1.1	19.1±.5	118±6

† one rat omitted from this group because it lost weight over the duration of the study.

* Significant Difference at $P < .05$.

Table 3.

Body Weight Measurements

SC-18862											
No. of Rats	Sex	Consumed (Mean \pm S.E.)	Body Weight (Gm) on Day							Mean \pm S.E. Weight Change (Day 7 - Day 0)	
		0	1	2	3	4	5	6	7		
10	Female	.152 \pm .003	195 \pm 3	198 \pm 3	202 \pm 3	206 \pm 4	209 \pm 4	211 \pm 4	213 \pm 4	217 \pm 5	22 \pm 2*
10	Female	Pair-Fed Control	193 \pm 2	197 \pm 2	198 \pm 3	197 \pm 3	202 \pm 3	204 \pm 3	205 \pm 3	206 \pm 2	13 \pm 1
10	Male	.200 \pm .005	154 \pm 2*	162 \pm 2	170 \pm 2	177 \pm 2	185 \pm 2	193 \pm 2	200 \pm 2	208 \pm 3	54 \pm 3*
10	Male	Pair-Fed Control	163 \pm 4	165 \pm 2	174 \pm 2	178 \pm 3	187 \pm 3	189 \pm 3	198 \pm 3	206 \pm 4	43 \pm 4
10	Female	1.48 \pm .07	191 \pm 2	194 \pm 3	197 \pm 2	199 \pm 2	202 \pm 2	206 \pm 2	207 \pm 2	210 \pm 2	19 \pm 2*
9 [†]	Female	Pair-Fed Control	190 \pm 5	190 \pm 6	194 \pm 5	195 \pm 4	198 \pm 4	199 \pm 4	200 \pm 5	202 \pm 4	13 \pm 2
10	Male	2.07 \pm .03	152 \pm 2	160 \pm 3	164 \pm 4	171 \pm 4	178 \pm 5	186 \pm 6	193 \pm 6	200 \pm 7	49 \pm 6
10	Male	Pair-Fed Control	156 \pm 4	162 \pm 3	170 \pm 3	176 \pm 4	183 \pm 4	187 \pm 5	196 \pm 5	200 \pm 4	44 \pm 2

[†] one rat omitted because of weight loss

* = Significant Difference at $P < .05$.

Table 4.

Water Consumption

No. of Rats		Sex	SC-18862 (Gm/Kg) Consumed (Mean \pm S.E.)	Water (ml) Consumed on Day					Weekly Water (ml) Intake (Mean \pm S.E.)		
				1	2	3	4	5	6	7	
10	Female		.152 \pm .003	22.4 \pm 2.3	22.9 \pm 2.6	23.1 \pm 3.1	24.3 \pm 2.8	22.3 \pm 2.3	22.8 \pm 2.2	24.4 \pm 3.0	197 \pm 3
10	Female	Pair-Fed Control		27.3 \pm 1.8	24.8 \pm 1.8	23.5 \pm 2.4	26.5 \pm 1.9	26.3 \pm 1.4	27.4 \pm 2.4	28.8 \pm 2.8	185 \pm 12
10	Male		.200 \pm .005	29.8 \pm 1.2	30.8 \pm 1.5	30.5 \pm 1.7	30.0 \pm 1.6	31.5 \pm 1.6	32.5 \pm 1.2	33.0 \pm 1.5	218 \pm 9
10	Male	Pair-Fed Control		29.0 \pm 2.4	30.8 \pm 1.6	28.1 \pm 1.7	31.7 \pm 2.0	28.7 \pm 1.1	33.1 \pm 1.6	32.5 \pm 3.2	214 \pm 10
10	Female		1.48 \pm .07	25.6 \pm 2.2	26.3 \pm 1.3	27.9 \pm 1.6	27.2 \pm 1.2	28.2 \pm 1.4	25.6 \pm 1.3	27.5 \pm 1.9	187 \pm 6
9 +	Female	Pair-Fed Control		25.2 \pm 2.9	29.2 \pm 3.0	25.6 \pm 2.6	29.1 \pm 1.9	27.4 \pm 1.5	25.8 \pm 2.4	28.7 \pm 2.4	191 \pm 14
10	Male		2.07 \pm .03	29.9 \pm .9	27.8 \pm 2.2	30.5 \pm 1.6	30.9 \pm 2.3	31.7 \pm 2.2	33.1 \pm 2.3	32.4 \pm 2.8	216 \pm 13
10	Male	Pair-Fed Control		28.8 \pm 1.9	30.1 \pm 1.6	29.9 \pm 1.9	29.1 \pm 1.7	28.7 \pm 2.4	31.2 \pm 1.6	31.3 \pm 1.7	209 \pm 11

+ one rat omitted because of weight loss.

* Significant Difference at $P < .05$.

Table 5.

Effect of SC-18862 on Cholesterol, Triglycerides and Free Fatty Acids in Serum

No. of Rats	Sex	SC-18862 (Gm/Kg) Consumed (Mean \pm S.E.)		Cholesterol (mg%) (Mean \pm S.E.)		Triglycerides (mg%) (Mean \pm S.E.)		Free Fatty Acids (μ moles/liter) (Mean \pm S.E.)	
10	Female	.152 \pm .003		62 \pm 3		78.5 \pm 17.5		325.6 \pm 33.1	
10	Female	Pair-Fed Control		59 \pm 2		57.9 \pm 8.4		299 \pm 28.3	
10	Male	.200 \pm .005		56 \pm 3		55.7 \pm 8.9		333.8 \pm 30.2	
10	Male	Pair-Fed Control		53 \pm 3		46.2 \pm 7.2		332.1 \pm 48.8	
10	Female	1.48 \pm .07		64 \pm 3		69.7 \pm 8.1		348.3 \pm 27.7	
9 [†]	Female	Pair-Fed Control		65 \pm 3		55.7 \pm 7.5		354.3 \pm 23.4	
10	Male	2.07 \pm .03		56 \pm 2		43.0 \pm 5.8		337.8 \pm 49.7	
10	Male	Pair-Fed Control		56 \pm 3		44.0 \pm 4.6		370.1 \pm 47.5	

[†] one rat omitted because of weight loss

* = Significant Difference at P < .05.

Table 6.
Effect of SC-18862 on Glucose and Insulin in Serum

No. of Rats	Sex	SC-18862 (Gm/Kg) Consumed (Mean \pm S.E.)	Glucose (mg%) (Mean \pm S.E.)	Insulin (μ gm/ml) (Mean \pm S.E.)
10	Female	.152 \pm .003	110 \pm 6	2.6 \pm .4
10	Female	Pair-Fed Control	117 \pm 9	2.8 \pm .3
10	Male	.200 \pm .005	130 \pm 5	4.3 \pm .9
10	Male	Pair-Fed Control	130 \pm 8	4.7 \pm 1.2
10	Female	1.48 \pm .07	113 \pm 7	3.1 \pm .4
9+	Female	Pair-Fed Control	114 \pm 6	2.5 \pm .3
10	Male	2.07 \pm .03	139 \pm 4	4.4 \pm .4
10	Male	Pair-Fed Control	140 \pm 7	3.1 \pm .5

* = Significant Difference at $P < .05$

+ = one rat omitted because of weight loss

Table 7.
Daily Amount of SC-19192 Consumed

No. of Rats	Sex	Anticipated SC-19192 Consumption	1	2	3	4	5	6	7	Mean (Gm/Kg) Daily Consumption (Mean±S.E.)
8+	Female	0.2 Gm/Kg	.135±.012	.149±.006	.180±.018	.165±.014	.160±.010	.140±.012	.143±.015	.153 ± .006
10	Male	0.2 Gm/Kg	.164±.012	.206±.015	.187±.009	.214±.016	.214±.009	.201±.014	.194±.011	.197 ± .007
10	Female	2 Gm/Kg	1.46 ± .09	1.46 ± .05	1.53 ± .13	1.69 ± .14	1.49 ± .12	1.57 ± .07	1.77 ± .10	1.57 ± .04
10	Male	2 Gm/Kg	1.56 ± .18	1.78 ± .11	1.87 ± .10	1.89 ± .08	1.92 ± .16	2.08 ± .08	2.10 ± .11	1.88 ± .07

+ - rat omission explained in text.

Table 8.

Food Consumption

No. of Rats		SC-19192 (Gm/Kg) Consumed (Mean±S.E.)	Weight (Gm) of Food Consumed on Day					Weekly Food Consumption (Gm) (Mean±S.E.)		
Sex		1	2	3	4	5	6	7		
8+	Female	.153 ± .006	12.8±1.2	14.0±.5	17.4±2.0	16.2±1.5	16.0±1.2	14.1±1.2	14.6±1.6	105 ± 5
7+	Female	Pair-Fed Control	11.8±.8	14.1±.5	17.3±1.0	13.7±1.7	14.4±1.4	14.6±1.2	15.7±1.1	102 ± 4
10	Male	.197 ± .007	13.4±1.0	17.4±1.1	16.3±.8	19.6±1.5	20.4±.8	19.8±1.5	19.1±1.5	127 ± 4
10	Male	Pair-Fed Control	13.9±1.0	17.2±.6	16.9±.8	18.4±.9	20.0±.7	19.5±1.3	19.1±.9	125 ± 3
10	Female	1.57 ± .04	13.9±.8	14.2±.6	15.0±1.3	16.7±1.2	15.1±1.2	16.0±.8	18.3±1.1	109 ± 3*
10	Female	Pair-Fed Control	10.4±1.5	12.4±1.3	13.4±1.1	14.5±.5	14.9±1.2	15.6±.6	16.9±.7	100 ± 3
10	Male	1.88 ± .07	12.7±1.6	15.1±.9	16.4±1.1	17.1±1.0	18.1±1.7	20.2±1.1	21.1±1.0	121 ± 7
10	Male	Pair-Fed Control	12.2±1.1	15.6±.8	16.3±1.0	17.9±1.0	17.2±1.5	19.0±1.2	19.9±.8	118 ± 6

† rat omission explained in text.

* = Significant Difference at $P < .05$.

Table 9.
Body Weight Measurements

SC-19192											
No. of Rats	Sex	Consumed (Mean \pm S.E.)	0	1	2	Body Weight (Gm) on Day (Mean \pm S.E.)				7	Mean \pm S.E. Weight Change (Day 7-Day 0)
						3	4	5	6		
8	Female	.153 \pm .006	187 \pm 4	189 \pm 4	189 \pm 4	192 \pm 5	196 \pm 5	200 \pm 5	201 \pm 5	203 \pm 5	17 \pm 3
7	Female	Pair-Fed Control	194 \pm 4	194 \pm 4	194 \pm 4	200 \pm 4	202 \pm 4	204 \pm 4	205 \pm 5	208 \pm 4	14 \pm 3
10	Male	.197 \pm .007	155 \pm 3	164 \pm 3	169 \pm 3	175 \pm 3	183 \pm 3	191 \pm 4	198 \pm 4	205 \pm 4	50 \pm 2*
10	Male	Pair-Fed Control	164 \pm 3	169 \pm 3	175 \pm 2	178 \pm 3	184 \pm 3	194 \pm 3	201 \pm 4	205 \pm 5	40 \pm 2
10	Female	1.57 \pm .04	188 \pm 2	191 \pm 2	194 \pm 2	195 \pm 3	198 \pm 3	202 \pm 2	204 \pm 2	207 \pm 2	19 \pm 2
10	Female	Pair-Fed Control	192 \pm 3	192 \pm 3	194 \pm 3	195 \pm 2	199 \pm 2	200 \pm 4	204 \pm 2	207 \pm 2	15 \pm 2
10	Male	1.88 \pm .07	153 \pm 4	161 \pm 3	169 \pm 4	174 \pm 4	180 \pm 4	187 \pm 4	193 \pm 5	201 \pm 5	48 \pm 5
10	Male	Pair-Fed Control	164 \pm 3	166 \pm 3	169 \pm 3	176 \pm 3	181 \pm 3	184 \pm 6	195 \pm 5	202 \pm 4	39 \pm 3

* = Significant Difference at P < .05.

Table 10.

Water Consumption

SC-19192										
No. of Rats	Sex	(Gm/Kg) Consumed (Mean \pm S.E.)	Water (ml) Consumed on Day					Weekly Water (ml) Intake (Mean \pm S.E.)		
		1	2	3	4	5	6	7		
8	Female	.153 \pm .006	25.2 \pm 1.7	25.2 \pm 2.4	26.2 \pm 1.8	28.6 \pm 1.8	26.8 \pm 1.3	25.5 \pm 2.5	27.9 \pm 3.0	186 \pm 8
7	Female	Pair-Fed Control	22.9 \pm 1.7	25.7 \pm 1.4	30.1 \pm 1.4	26.9 \pm 2.2	27.7 \pm 1.1	27.6 \pm 2.4	30.3 \pm 2.1	191 \pm 10
10	Male	.197 \pm .007	26.0 \pm 1.6	30.5 \pm 1.0	31.1 \pm 1.0	33.8 \pm 1.1	34.2 \pm .9	33.9 \pm 1.3	34.1 \pm 1.5	184 \pm 14
10	Male	Pair-Fed Control	28.8 \pm 1.5	33.2 \pm 2.3	30.5 \pm 1.9	33.1 \pm 2.3	34.9 \pm 1.5	34.7 \pm 2.2	32.5 \pm 3.1	219 \pm 13
10	Female	1.57 \pm .04	29.3 \pm 1.6	29.2 \pm 1.7	31.4 \pm 1.9	32.2 \pm 1.7	31.9 \pm 1.8	31.1 \pm 1.1	33.3 \pm 1.7	219 \pm 7*
10	Female	Pair-Fed Control	23.2 \pm 1.3	25.6 \pm 1.5	24.2 \pm 2.0	27.2 \pm .8	27.0 \pm 2.5	29.5 \pm .8	29.4 \pm 1.4	186 \pm 7
10	Male	1.88 \pm .07	31.9 \pm 3.9	30.1 \pm 1.6	32.3 \pm 2.1	34.3 \pm 1.7	35.8 \pm 1.6	36.9 \pm 2.4	38.6 \pm 2.3	240 \pm 12*
10	Male	Pair-Fed Control	21.7 \pm 1.6	28.2 \pm 1.2	28.8 \pm 1.2	31.5 \pm 1.7	28.4 \pm 1.9	34.5 \pm 1.6	32.9 \pm 1.8	206 \pm 7

* = Significant Difference at P < .05.

Table 11.

Effect of SC-19192 on Cholesterol, Triglycerides and Free Fatty Acids in Serum

No. of Rats	Sex	SC-19192 (Gm/Kg) Consumed (Mean \pm S.E.)		Cholesterol (mg%) (Mean \pm S.E.)		Triglycerides (mg%) (Mean \pm S.E.)		Free Fatty Acids (μ moles/liter) (Mean \pm S.E.)	
8	Female	.153 \pm .006		56 \pm 2		50.0 \pm 6.9		382.2 \pm 57.4	
7	Female	Pair-Fed Control		53 \pm 2		44.8 \pm 6.1		414.1 \pm 47.4	
10	Male	.197 \pm .007		56 \pm 3		67.6 \pm 7.5		317.7 \pm 16.5	
10	Male	Pair-Fed Control		55 \pm 3		54.0 \pm 9.1		470.7 \pm 78.5	
10	Female	1.57 \pm .04		58 \pm 3		41.8 \pm 4.2		391.8 \pm 44.6	
10	Female	Pair-Fed Control		63 \pm 3		41.8 \pm 5.7		334.5 \pm 22.9	
10	Male	1.88 \pm .07		56 \pm 2		60.5 \pm 9.9		333.3 \pm 28.1	
10	Male	Pair-Fed Control		52 \pm 2		53.2 \pm 7.2		321.4 \pm 43.1	

* = Significant Difference at P < .05.

Table 12.
Effect of SC-19192 on Glucose and Insulin in Serum

No. of Rats	Sex	SC-19192 (Gm/Kg) Consumed (Mean \pm S.E.)	Glucose (mg%) (Mean \pm S.E.)	Insulin (μ gm/ml) (Mean \pm S.E.)
8	Female	.144 \pm .015	148 \pm 11	2.2 \pm .4
7	Female	Pair-Fed Control	155 \pm 9	2.1 \pm .3
10	Male	.192 \pm .014	137 \pm 4	2.8 \pm .9
10	Male	Pair-Fed Control	123 \pm 7	3.0 \pm .8
10	Female	1.54 \pm .15	144 \pm 9	1.8 \pm .3
10	Female	Pair-Fed Control	141 \pm 9	1.5 \pm .3
10	Male	1.97 \pm .13	133 \pm 4	3.1 \pm .9
10	Male	Pair-Fed Control	140 \pm 7	3.2 \pm .9

* = Significant Difference at $P < .05$

Table 13.
Daily Amount of SC-18862 Consumed

No. of Rats	Sex	Anticipated SC-18862 Consumption	1	2	3	4	5	6	7	Mean (Gm/Kg) Daily Consumption (Mean S.E.)
10	Female	2.0 Gm/Kg	1.72 ± .07	2.16 ± .16	2.13 ± .07	2.21 ± .08	2.01 ± .08	2.00 ± .06	2.01 ± .09	2.03 ± .06

Table 14.
Food Consumption

SC-18862		Weight (Gm) of Food Consumed on Day							Weekly Food
No. of Rats	Sex	(Gm/Kg) Consumed (Means S.E.)	1	2	3	4	5	6	(Gm) Consumption (Means S.E.)
10	Female	2.03 ± .06	11.4± .5	14.7±1.1	14.7± .6	15.5± .6	14.3± .6	14.5± .5	14.9± .7
10	Female	Ad libitum Control	13.3± .7	15.3± .4	15.2± .7	15.6± .6	15.4± .6	15.5± .7	16.0± .8

* Significant Difference at $P < .05$.

Table 15.

Body Weight Measurements

SC-18862												
No. of Rats	Sex	Consumed (Mean \pm S.E.)	0	1	2	Body Weight (Gm) on Day (Mean \pm S.E.)				6	7	Mean \pm S.E. Weight Change (Day 7-Day 0)
10	Female	2.03 \pm .06	176 \pm 2	179 \pm 2	183 \pm 2	186 \pm 2	189 \pm 2	192 \pm 2	195 \pm 2	200 \pm 2	23 \pm 2	
10	Female	Ad libitum										
	Control	184 \pm 2	187 \pm 2	190 \pm 2	194 \pm 2	196 \pm 2	199 \pm 2	204 \pm 2	206 \pm 2	22 \pm 1		

* = Significant Difference at P < .05.

Table 16.

Water Consumption

No. of Rats	Sex	SC-18862 (Gm/Kg) Consumed (Mean \pm S.E.)	Water (ml) Consumed on Day					Weekly Water (ml) Intake (Mean \pm S.E.)		
			1	2	3	4	5		6	7
10	Female	2.03 \pm .06	25.1 \pm 1.1	29.9 \pm 1.6	28.1 \pm 1.0	29.2 \pm .6	27.9 \pm .9	29.1 \pm .9	29.1 \pm 1.2	197 \pm 5
10	Female	Ad libitum Control	29.4 \pm 1.2	30.1 \pm 1.2	30.6 \pm 1.6	29.2 \pm 1.5	29.7 \pm 1.0	30.7 \pm 1.6	31.0 \pm 1.8	211 \pm 7

* = Significant Difference at $P < .05$.

Table 18.

Effect of SC-18862 on Glucose and Insulin in Serum

No. of Rats	Sex	SC-18862 (Gm/Kg) Consumed (Mean \pm S.E.)	Glucose (mg%) (Mean \pm S.E.)	Insulin (μ gm/ml) (Mean S.E.)
10	Female	2.03 \pm .06	148 \pm 4	3.1 \pm .4
10	Female	Ad libitum Control	140 \pm 12	2.5 \pm .3

* Significant Difference at $P < .05$.