(2) "The National Formulary," 12th ed., Mack Publishing Co., Easton, Pa., 1965, p. 7.
(3) "Remington's Pharmaceutical Sciences," 13th ed., Mack Publishing Co., Easton, Pa., 1965, p. 518.
(4) D.P.S.C. Standards for the Manufacture and Packaging of Drugs, Pharmaceuticals and Biological Products, Defense Personnel Support Center, Philadelphia, PA 19101, Sept. 1968, Appendix II, p. 52.
(5) C. F. Stafficker, *Bull. Parenteral Drug Ass.*, **10**, 10(1956).
(6) W. J. Artz, W. T. Gloor, Jr., and D. R. Reese, *J. Pharm. Sci.*, **50**, 258(1961).
(7) A. N. Martin, "Physical Pharmacy," Lea & Febiger, Philadelphia, Pa., 1960, p. 533.
(8) R. R. Macintosh, "Lumbar Puncture and Spinal Analgesia," Williams & Wilkins, Baltimore, Md., 1951, p. 143.
(9) R. W. Cope, F. Prescott, and T. D. Whittet, *Pharm. J.*, **114**, 222(1952).
(10) B. L. Steinberg, *Anesthesiology*, **11**, 257(1950).
(11) T. DeForest, U. S. pat. 2,472,522 (June 7, 1949).
(12) E. Völcker, *Z. Naturforsch.*, **6a**, 512(1951).

ACKNOWLEDGMENTS AND ADDRESSES

Received February 28, 1972, from the *Pharmaceutical Research and Development Laboratories of Merrell-National Laboratories, Division of Richardson-Merrell, Inc., Cincinnati, OH 45215*

Accepted for publication June 1, 1972.

* Present address: School of Pharmacy, University of Wisconsin, Madison, WI 53706

▲ To whom inquiries should be directed.

Acute and Chronic Effects of 3,4-Dimethoxyphenylacetamide on Plasma Glucose and Cholesterol in Rats

EDWARD C. KRIMMER, HERBERT BARRY, III▲, and JOSEPH P. BUCKLEY

Abstract □ One hour after a single intraperitoneal dose of 50–200 mg./kg. of 3,4-dimethoxyphenylacetamide to satiated and 24-hr. fasted rats, plasma glucose was markedly increased but plasma cholesterol was reliably decreased only by the highest dose in one of two studies with satiated animals. At 24 hr. after the 10th daily oral drug administration, plasma cholesterol of rats was unaffected by 3,4-dimethoxyphenylacetamide (100 mg./kg.) but markedly decreased by a standard hypocholesterolemic agent (20,25-diazacholesterol). An acute, toxic effect of 3,4-dimethoxyphenylacetamide was indicated by a marked decrease in spontaneous motor activity and in body weight gain. Contrary to a previous suggestion, 3,4-dimethoxyphenylacetamide does not appear to be a promising hypocholesterolemic agent; observations of acute hyperglycemia are at least partly attributable to the stressful effects of the toxic doses given.

Keyphrases □ 3,4-Dimethoxyphenylacetamide—hypocholesterolemic and hyperglycemic activity, compared to 20,25-diazacholesterol, rats □ Hyperglycemic activity—3,4-dimethoxyphenylacetamide □ Hypocholesterolemic agents, potential—3,4-dimethoxyacetamide, compared to 20,25-diazacholesterol

A previous article from these laboratories (1) reported the pharmacological evaluation of 3,4-dimethoxyphenylacetamide. The crystalline compound was isolated from the leaves of *Catharantus lanceus* and later synthesized (2). A possible therapeutic use of this compound was suggested by structural similarities to phenylethylacetic acid, a compound shown to have hypocholesterolemic properties (3). Sofia *et al.* (1), in a test for this effect at 30 and 60 min. after administration of a single dose (100 mg./kg.) to rats fasted for 24 hr., reported a significant reduction of plasma cholesterol at the 60-min. postinjection time, with no change in plasma glucose levels. A test of spontaneous activity in mice showed marked CNS depression with doses as low as 6.25 mg./kg.

The first experiment reported in the present paper evaluates the acute effects of 3,4-dimethoxyphenylacetamide, repeating the previous studies on the effects of the compound on plasma glucose and cholesterol levels (1). In addition, fasted and satiated rats were compared because this variable may be important with oral administration of the drug. The effect of the compound on spontaneous activity was determined in the same animals, immediately preceding the test for plasma glucose and cholesterol.

Most references on hypocholesterolemic compounds indicate that chronic, oral administration is used for lowering the plasma cholesterol (4–8). Therefore, the present paper reports a second experiment comparing acute oral with intraperitoneal effects of 3,4-dimethoxyphenylacetamide and a third experiment comparing chronic oral effects of this compound with 20,25-diazacholesterol dihydrochloride[1], a standard hypocholesterolemic agent (8), both administered for 10 days prior to determination of plasma glucose and cholesterol. In this chronic study, hypercholesterolemia was induced in some animals by concurrent administration of propylthiouracil in the drinking fluid.

[1] SC-12937.

Table I—Square Root Activity Counts (Mean and *SE*) between 50 and 60 min. after a Single Dose of 3,4-Dimethoxyphenylacetamide or Its Vehicle

	Vehicle	Dosage, mg./kg. 50	100	200
Satiated group[a]				
Mean	32.3	15.0[b]	8.2[b]	21.9[c]
SE	1.11	2.08	4.10	2.28
N	3	3	3	3
Fasted group				
Mean	21.8	9.8[c]	9.1[b]	6.3[b]
SE	3.96	0.93	0.65	1.51
N	4	5	5	4

[a] One of the four animals in each satiated group was omitted because of malfunction of the recording elements. [b] $p < 0.01$ for difference from vehicle. [c] $p < 0.05$ for difference from vehicle.

EXPERIMENTAL

Animals—Male, albino rats[2], Wistar descendants weighing 150–175 g. at the time of receipt, were housed in individual cages with the environmental temperature controlled at 22 ± 1° and an automatically regulated cycle of 12 hr. light, 12 hr. dark. Experimental procedures were begun after 5–7 days of acclimation to the housing conditions. Food and water were continuously available except as specified for Experiment I.

Procedures—All tests and drug administrations were during the lighted portion of the daily cycle. Experiment I was conducted on two groups of rats: 16 satiated and 18 24-hr. fasted at the time of the test. Four animals in each group were randomly assigned to each of four dosage conditions (3,4-dimethoxyphenylacetamide: 50, 100, and 200 mg./kg. and its vehicle), except for five fasted animals given 50 and 100 mg./kg. The compound (50, 100, and 200 mg./kg.) or its vehicle (0.25% agar suspension) was administered intraperitoneally in a volume of 4 ml./kg. body weight, followed by placement of the animals in individual waiting cages without food or water. Blood samples for measuring plasma glucose and cholesterol (9) were collected by decapitation, 60 min. after injection. Spontaneous motor activity was recorded in an Actophotometer (10) for each animal for 10 min. immediately preceding decapitation. The time interval was less than 1 min. between removal of the animal from the Actophotometer and completion of blood collection.

Blood samples were centrifuged at 2300×*g* for 10 min., and plasma was collected and frozen. Plasma glucose and cholesterol determinations were made within 24 hr. of collection using a colorimetric technique[3].

In Experiment II, the same procedures as in Experiment I were applied to 24 satiated animals, divided randomly into four groups of six each. The 3,4-dimethoxyphenylacetamide (200 mg./kg.) was administered orally to one group and intraperitoneally to another. Controls were provided by oral and intraperitoneal administration of the vehicle to the other two groups.

In Experiment III, 30 animals were divided randomly into two equal groups, one receiving propylthiouracil (0.01%) and the other tap water as their drinking fluid. Both groups were subdivided into three groups of five animals each, receiving orally 3,4-dimethoxyphenylacetamide (100 mg./kg.), 20,25-diazacholesterol dihydrochloride (3.0 mg./kg.), or the same vehicle as in Experiments I and II in a volume of 3 ml./kg. for 10 days. All drinking fluids were replaced with fresh ones on alternate days. Body weight and consumption of food and fluid were recorded daily. At 24 hr. after the 10th daily treatment, plasma glucose and cholesterol were collected and measured as in Experiment I.

Drug Preparation—*For the satiated animals in Experiment I*, the 3,4-dimethoxyphenylacetamide and agar were pulverized together and sufficient distilled water was added to produce a mixture of 200 mg./ml. with 0.25% agar in water. The combination was slowly heated to permit the compound to form a suspension in the agar. Further dilution from the stock was made by adding 0.25% agar suspension. For the fasted animals in Experiment I, and throughout Experiments II and III, the 3,4-dimethoxyphenylacetamide was powdered and a sufficient quantity of 0.25% agar was slowly added to produce the stock suspension from which further dilutions were made. This modification, which replicated the procedure used by Sofia *et al.* (1), was designed to prevent any possible degradation of the compound due to heating.

[2] The animals were obtained from Hilltop Lab Animals, Inc., Scottdale, Pa., for Experiment I and from Zivic-Miller, Inc., Pittsburgh, Pa., for Experiments II and III.

[3] Outlined in the Dow Diagnostest brochure.

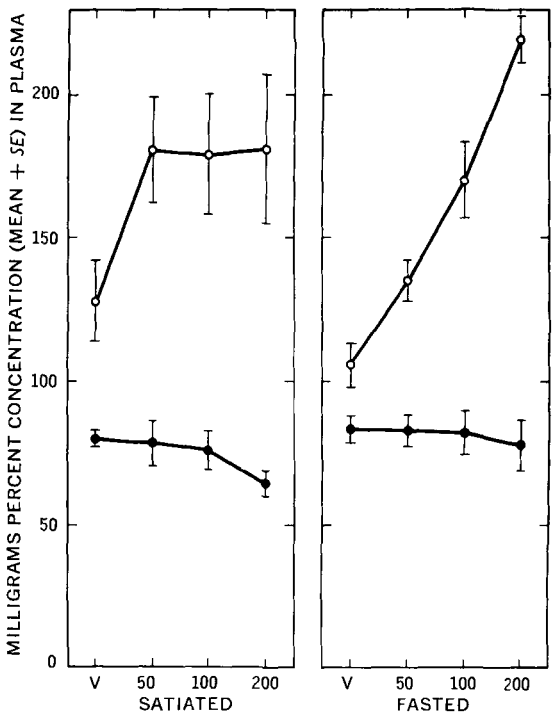

Figure 1—*Plasma glucose* (○) *and cholesterol* (●) *at 60 min. after intraperitoneal injection of 50, 100, or 200 mg./kg. of 3,4-dimethoxyphenylacetamide or its vehicle* (*V*) *in satiated and fasted rats.*

RESULTS

Figure 1 shows the acute effects of a single dose of 3,4-dimethoxyphenylacetamide on plasma cholesterol and plasma glucose at the 1-hr. interval (Experiment I). The drug had little effect on plasma cholesterol, although the levels were progressively lower with increasing doses of the compound; the difference from the vehicle condition reached statistical significance for the highest dose (200 mg./kg.) in the satiated animals ($t = 2.99$, $df = 6$, $p < 0.05$). The same treatments caused substantial increases in plasma glucose levels. For the fasted animals, the increase was monotonically related to the dose of the compound, with each of the three doses differing reliably from the vehicle. For the satiated animals, a substantial, similar elevation of plasma glucose was found with all three dosages. None of the individual drug groups differed reliably from the vehicle group, but a statistically significant elevation was shown for the 12 drug animals, combining the three doses ($t = 2.41$, $df = 14$, $p < 0.05$).

The glucose values were less variable among the animals in each fasted group than among those in the corresponding satiated groups, as shown by the smaller standard errors, so that the relatively small increase in plasma glucose for the fasted animals after the lowest dose of 3,4-dimethoxyphenylacetamide (50 mg./kg.) was statistically reliable. Comparison between the satiated and fasted vehicle groups (Fig. 1) shows virtually no difference in plasma cholesterol. The fasted vehicle animals had lower glucose levels than the satiated vehicle animals, in accordance with the effect to be expected from 24 hr. of food deprivation, but the difference was not statistically significant.

The effects of the compound on spontaneous motor activity are shown in Table I. All three doses had a large and reliable depressant effect, with a monotonic dose–response function for the fasted but not the satiated animals.

Table II—Mean and Standard Error of Plasma Cholesterol and Glucose at 60 min. and of Square Root Activity Counts at 50–60 min. after Intraperitoneal or Oral Administration of 3,4-Dimethoxyphenylacetamide (200 mg./kg.) or Its Vehicle

	Oral		Intraperitoneal	
	Vehicle	Drug	Vehicle	Drug
Number of animals	6	6	6	6
Plasma cholesterol				
Mean	65.4	70.5	67.7	66.2
SE	4.73	4.50	6.81	4.77
Plasma glucose				
Mean	152.8	171.6	157.6	233.4[a]
SE	5.87	9.64	4.38	17.89
Square root activity counts				
Mean	19.9	11.4[a]	17.2	7.5[a]
SE	1.90	1.65	1.11	1.98

[a] $p < 0.01$ for difference from vehicle for same route of administration.

The results of Experiment II are summarized in Table II. The plasma cholesterol showed a negligible difference between the drug and vehicle groups, both with oral and intraperitoneal administration. The plasma glucose was reliably elevated by the drug administered intraperitoneally ($t = 4.12$, $df = 10$, $p < 0.01$). The same tendency was found with the oral route, but the difference from the vehicle group was short of statistical significance. Spontaneous motor activity was reliably decreased by the drug administered orally ($t = 3.41$, $df = 10$, $p < 0.01$) and intraperitoneally ($t = 4.24$, $df = 10$, $p < 0.01$).

Figure 2 summarizes, for Experiment III, the plasma glucose and cholesterol levels at 24 hr. after the 10th daily oral dose of 3,4-dimethoxyphenylacetamide, 20,25-diazacholesterol (a standard hypocholesterolemic agent), or the vehicle. The standard hypocholesterolemic agent reliably decreased the cholesterol level below the vehicle controls ($t = 2.73$, $df = 8$, $p < 0.05$ for the animals given water; $t = 3.94$, $df = 8$, $p < 0.01$ for those given propylthiouracil as their drinking fluid). The propylthiouracil treatment increased cholesterol in all groups, as would be expected, but the difference was statistically reliable only for the 3,4-dimethoxyphenylacetamide group ($t = 2.97$, $df = 8$, $p < 0.05$).

The glucose levels were closely similar for all of the groups shown in Fig. 2, with the exception of the elevated average in the water-treated 3,4-dimethoxyphenylacetamide group. Its difference from the water-treated vehicle group approached the conventional 5% criterion for statistical reliability ($t = 2.09$, $df = 8$, $p < 0.10$).

Toxic effects of 3,4-dimethoxyphenylacetamide were seen in the first 24 hr. after the first oral administration of this compound, as shown by changes in body weight, compared with the vehicle group under the same propylthiouracil or water condition (Table III). Furthermore, adverse effects of adding propylthiouracil to the drinking fluid in the first 24 hr. were shown by comparison with animals drinking water for the same 3,4-dimethoxyphenylacetamide or vehicle group. However, the standard hypocholesterolemic compound, which had no reliable effect on any of the measures in the animals receiving tap water, counteracted the adverse effects of propylthiouracil on weight gain during the first 24 hr. of these combined treatments (Table III). These differences among groups in weight change are at least partly attributable to differences in food and fluid intake. The 3,4-dimethoxyphenylacetamide reliably decreased fluid intake ($t = 2.45$, $df = 28$, $p < 0.05$) and food intake ($t = 2.97$, $df = 28$, $p < 0.01$) when this compound was compared with the standard hypocholesterolemic agent and the vehicle, pooled together, with the water and propylthiouracil conditions also being pooled together.

DISCUSSION

Contrary to the suggestion by Sofia *et al.* (1), 3,4-dimethoxyphenylacetamide does not seem likely to be clinically useful as a hypocholesterolemic agent; it does not have the necessary attributes

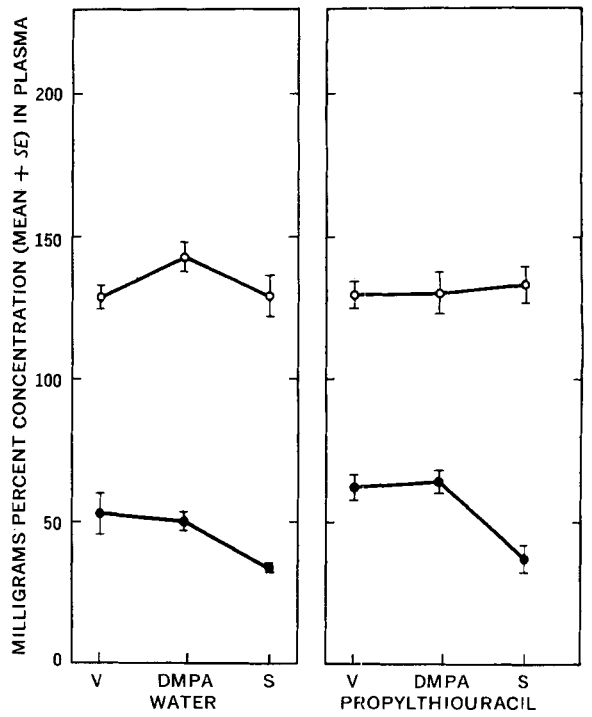

Figure 2—*Plasma glucose* (○) *and cholesterol* (●) *at 24 hr. after the 10th daily oral administration of 100 mg./kg. of 3,4-dimethoxyphenylacetamide (DMPA), the standard hypocholesterolemic agent, 20,25-diazacholesterol (S), or the vehicle (V) in rats given water or propylthiouracil as their drinking fluid.*

of a specific effect, after chronic administration, at doses that are not physiologically or behaviorally toxic. A hypocholesterolemic effect of this compound has been demonstrated, but only acutely, at the high doses of 200 mg./kg. for rats (Fig. 1) and 100 mg./kg. for mice (1). These far exceed the doses found sufficient to depress spontaneous activity: 50 mg./kg. in rats (Table I) and 6.25 mg./kg. in mice (1). The failure of chronic 3,4-dimethoxyphenylacetamide treatment to reduce cholesterol (Experiment III) indicates that the hypocholesterolemic effect of this compound is only acute and not cumulative, even when plasma cholesterol was elevated by propylthiouracil treatment. The results of Experiment II indicate that 3,4-dimethoxyphenylacetamide is adequately absorbed after oral administration, although the drug effects are weaker, as would be expected, than at the same 1-hr. interval after intraperitoneal administration. Moreover, responsiveness to a hypocholesterolemic drug

Table III—Effects of the First 24 hr. of Propylthiouracil Added to the Drinking Fluid and Daily Oral 3,4-Dimethoxyphenylacetamide or the Standard Hypocholesterolemic Agent on Change in Body Weight and in Fluid and Food Intake (Mean ± *SE*) in Comparison with the Preceding and Subsequent 24-hr. Periods Averaged Together

Drug Condition	Body Weight, g.	Fluid Intake, ml.	Food Intake, g.
Drinking Fluid: Propylthiouracil			
3,4-Dimethoxyphenylacetamide	−2.8 ± 2.5	−6.9 ± 2.5	−3.4 ± 1.4
Standard	0.7 ± 1.1[a]	−2.3 ± 1.5	−0.4 ± 0.6
Vehicle	−4.4 ± 1.5[b]	−7.1 ± 1.9	−1.6 ± 0.4
Drinking Fluid: Water			
3,4-Dimethoxyphenylacetamide	−3.9 ± 1.9[a]	−10.6 ± 3.8	−3.4 ± 1.6
Standard	0.3 ± 1.8	−4.5 ± 1.2	−1.0 ± 0.8
Vehicle	1.0 ± 0.8	−1.8 ± 1.5	−1.1 ± 0.6

[a] $p < 0.05$ for difference from vehicle for same propylthiouracil or water treatment. [b] $p < 0.05$ for difference from water for same drug condition.

was clearly shown by the lower cholesterol levels after 10 days of treatment with 20,25-diazacholesterol (Fig. 2). The cholesterol values for the standard drug and vehicle groups after propylthiouracil treatment were closely similar to those reported by Ranney and Cook (8). The present experiment also gives evidence for a short-term therapeutic action of 20,25-diazacholesterol in reversing adverse effects of propylthiouracil during the first 24 hr. of treatment (Table III).

The principal physiological effect of 3,4-dimethoxyphenylacetamide was the glucose elevation at 1 hr. after a single dose of this compound. Since the blood collection was completed in less than 1 min., this procedure probably did not elevate plasma glucose (11), thus enabling better differentiation between the drug and vehicle groups in this respect. Hyperglycemia is one of the manifestations of a stress response (11, 12), which may also have been evidenced by a severe decrease in the behavioral measure of spontaneous motor activity (Experiments I and II) and by adverse effects during 24 hr. on body weight (Experiment III). In Experiment I, labored respiration was observed within 20 min. after administration of 3,4-dimethoxyphenylacetamide. In addition to the possible stress-induced glycolysis, which could account for hyperglycemia in satiated animals, the large and reliable glucose elevation in fasted animals suggests that gluconeogenesis also contributed to the hyperglycemic response to this compound, because the glycogen stores of the liver should be depleted after 24 hr. of fasting. The tendency for hyperglycemia in the water group of Experiment III (shown in Fig. 2) gives evidence that some degree of hyperglycemia persists for as long as 24 hr. after administration of this compound.

The hypocholesterolemia at 1 hr. after a single dose of 3,4-dimethoxyphenylacetamide, reported previously (1), was found in the satiated animals in Experiment I but not in satiated animals received from a different supplier (Experiment II). The present results do not enable us to identify the conditions that determine this effect of the compound. The present series of experiments consistently showed a hyperglycemic effect of 3,4-dimethoxyphenylacetamide, which was not found by Sofia *et al.* (1). Their plasma glucose levels were considerably lower than the corresponding ones found in the present study and generally reported by other investigators. Since their animals were tested as early as 3 days after arrival in the laboratory, the 24-hr. fasting might have had a more severe effect, resulting in lower levels of plasma glucose. A suggestive parallel to these seemingly contradictory findings is found in effects of ethyl alcohol. A stress-induced hyperglycemic or hypoglycemic response may occur, depending on a number of variables, including the animal's nutritional condition and the time interval after alcohol administration (13).

REFERENCES

(1) R. D. Sofia, A. B. Segelman, N. R. Farnsworth, and J. P. Buckley, *J. Pharm. Sci.*, **60,** 1240(1971).

(2) A. B. Segelman, N. R. Farnsworth, H. H. S. Fong, and D. J. Abraham, *Lloydia*, **33,** 25(1970).

(3) T. Chrusciel, *Arch. Immunol. Ter. Dosw.*, **6,** 713(1958).

(4) T. S. Danowski, J. H. Sunder, D. G. Corredor, Y. Jung, J. W. Vester, R. C. Khurana, and J. P. Wingert, *Clin. Pharmacol. Ther.*, **12,** 126(1971).

(5) G. S. Boyd and M. F. Oliver, *J. Endocrinol.*, **21,** 25(1960).

(6) R. E. Counsell, P. D. Klimstra, R. E. Ranney, and D. L. Cook, *J. Med. Pharm. Chem.*, **5,** 720(1962).

(7) M. M. Best and C. H. Duncan, *Atherosclerosis*, **12,** 185 (1970).

(8) R. E. Ranney and D. L. Cook, *Arch. Int. Pharmacodyn. Ther.*, **154,** 51(1965).

(9) J. Cottet, A. Mathivat, and J. Redel, *Presse Med.*, **62,** 939 (1954).

(10) N. Watzman, H. Barry, III, W. J. Kinnard, Jr., and J. P. Buckley, *J. Pharm. Sci.*, **55,** 518(1966).

(11) E. L. Besch and B. J. Chou, *Proc. Soc. Exp. Biol. Med.*, **138,** 1019(1971).

(12) H. Barry, III, and J. P. Buckley, *J. Pharm. Sci.*, **55,** 1159 (1966).

(13) H. Wallgren and H. Barry, III, "Actions of Alcohol," vol. 1, Elsevier, Amsterdam, London, and New York, 1970, p. 110.

ACKNOWLEDGMENTS AND ADDRESSES

Received February 22, 1972, from the *Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15213*

Accepted for publication June 14, 1972.

Supported by Public Health Service Training Grant GM-1217 from the National Institute of General Medical Sciences and by Public Health Service Research Grant MH-13595 and Research Scientist Development Award K2-MH-5921, both from the National Institute of Mental Health to H. Barry, III.

Acknowledgment is gratefully made to Dr. Alvin B. Segelman of Rutgers University School of Pharmacy for supplying the 3,4-dimethoxyphenylacetamide and to G. D. Searle & Co., Chicago, Ill., for supplying the 20,25-diazacholesterol.

▲ To whom inquiries should be directed.