TABLE III. Effect of Bile Salt on Triton Inhibition.

Supplement	Change in lipolytic activity with added supplements: None	Bile salt .2%	Triton .03%	Bile and Triton
μg/ml*	53	81	29	28
%	100	153	55	53

* Amount of glycerol freed during incubation without and with the various supplements.

Although this decrease in absorption does occur, some fat is absorbed as evidenced by the appearance of radioactivity in the blood after ingestion of labeled fat. Since the presence of bile acids in the intestine might alter conditions, the possibility that their presence might prevent some of the inhibitory action of Triton on fat absorption was investigated. The bile acid (0.2%) was found to stimulate lipolytic activity while a combination of both bile acid and Triton (0.03%) had the same inhibitory effect as Triton alone (Table III).

If Triton is absorbed after ingestion of massive amounts (1 g per kg), its presence in the blood should inhibit *in vitro* lipolysis since only trace amounts are required for this action. The serums from each of the 5 rats had no inhibitory action on the activity of pancreatic lipase when tested for *in vitro*. This evidence supports both the finding of Cornforth *et al*(3), and our failure to obtain an elevated lipid level after ingestion of Triton alone(6), that such Triton is not absorbed (Table I).

Summary. The effect of oral ingestion of Triton on fat absorption in rats was investigated by feeding either fat or Triton alone, or a combination of fat and Triton. Serum esterified fatty acid levels were elevated after the fat feeding but not in either case after Triton ingestion. An attempt to account for this decreased fat absorption was made by determining the effect on the lipolytic activity of pancreatic lipase of Triton, alone and in the presence of a bile acid. A marked inhibition of lipolysis occurred in both cases when Triton was present. The absence of any inhibitory effect of blood serum on lipase activity after the ingestion of massive amounts of Triton indicated that the Triton was not absorbed.

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Restoration of Tyramine Pressor Responses in Reserpine-Treated Animals by Methyldopa and Its Amine Metabolites. (30025)

WILLIAM A. PETTINGER,* SYDNEY SPECTOR, DAVID HORWITZ AND ALBERT SJOERDSMA
Experimental Therapeutics Branch, National Heart Institute, Bethesda, Md.

The hypotensive agents, reserpine and methyldopa (L-α-methyldopa, Aldomet®), are known to lower tissue levels of norepinephrine in experimental animals(1,2), thereby suggesting similar mechanisms of action on the sympathetic nervous system. While reduced norepinephrine levels have been found in surgical biopsies of atrial appendages from patients receiving reserpine(3), corresponding data are not available in man for methyldopa. Since pressor responses to tyramine are diminished after depletion of norepinephrine stores by reserpine(4), responsiveness to tyramine has been used as a

* Present address: Dept. of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.

pharmacologic means of detecting depletion of norepinephrine from sympathetic nerve endings. Pressor responses to tyramine are markedly diminished in patients receiving reserpine(5,6); in contrast, we have found (6) as have Dollery *et al*(7) that during treatment with methyldopa responses to tyramine are not inhibited but rather may actually be enhanced. Furthermore, administration of methyldopa results in a restoration of responsiveness to tyramine in patients receiving reserpine(6). The present studies were designed to explore further in reserpinized animals this interesting and apparently paradoxic effect of methyldopa. The findings in patients were confirmed in observations on both dogs and rabbits; also, suggestive evidence was obtained that α-methylnorepinephrine may play a predominant role in enhancement of tyramine sensitivity following methyldopa treatment. While this paper was in preparation, similar results with methyldopa treatment in cats were reported from 2 other laboratories(8,9).

Methods. Studies were performed in mongrel dogs (7.6-21 kg) and albino rabbits (1.7-2.9 kg) under anesthesia with pentobarbital. Both vagus nerves were cut and the trachea was cannulated for maintenance of respiration with a Harvard respirator pump. Arterial blood pressure was measured continuously with a Statham pressure transducer through a femoral or carotid cannula and recorded on a Grass Polygraph recorder. An indwelling catheter was inserted in a femoral vein for intravenous injections.

On the day prior to study some of the animals were injected with reserpine given in 2 divided doses separated by an 8- to 12-hour interval; the dogs received a total dose of 0.8 mg/kg intramuscularly and the rabbits 2 to 6 mg/kg intravenously. Reserpine was prepared for injection by dissolving the free base in a few drops of glacial acetic acid and adding 0.5 ml of propylene glycol, 0.5 ml of ethanol and 2 ml of distilled water.

On the day of study control determinations of pressor responses to intravenous doses of tyramine hydrochloride were performed and the maximum dose which produced a rise of systolic blood pressure enhancement of pressor responses to tyramine by various agents. The compounds were administered either by rapid injection or infusion over a 10-minute period in the following doses (as free amino acid or base): D or L-α-methyldopa, 1.0 g in dogs and 75 mg/kg in rabbits; DL-α-methyldopamine hydrochloride, 2 mg/kg in rabbits and 0.2 mg/kg in dogs; L-α-methylnorepinephrine and L-norepinephrine bitartrate, 2 μg/kg in dogs and 6 μg/kg in rabbits.

Doses of the various test compounds were not repeated until effects of prior doses had dissipated. In the comparison of isomers of α-methyldopa, administration of the inactive D-isomer always preceded that of the L-isomer. Additional descriptions of the studies will be found in appropriate portions of the section on *Results.*

Results. Responses to tyramine after pretreatment with reserpine. Maximum doses of tyramine which were without pressor effectiveness were established in 18 dogs previously treated with reserpine. Such maximum ineffective doses ranged from 60 to 160 μg/kg and were found to average 96 μg/kg. Comparable findings in 4 untreated dogs were 7 to 24 μg/kg with an average of 15 μg/kg. Similar observations in rabbits revealed maximum ineffective doses of tyramine which averaged 226 μg/kg (100 to 390 μg/kg) after reserpine and averaged 42 μg/kg (26 to 88 μg/kg) in untreated animals. Markedly reduced sensitivity to the pressor effects of tyramine after reserpine was thus evident in the dogs and rabbits studied.

Effects of methyldopa. When 7 of the reserpine-treated dogs received methyldopa, 1.0 g intravenously, progressive increases in pressor responses to tyramine occurred reaching a peak in 1 to 2 hours (Fig. 1) persisting at this level for approximately 3 hours and declining thereafter; generally, some residual effects could still be observed after 8 hours when the studies were terminated. Comparable effects on blood pressure responses to tyramine were seen in 4 reserpine-treated rabbits which received 75 mg/kg doses of methyldopa (Fig. 2). In contrast 4 dogs and 2 rabbits which had not received reserpine pretreatment showed no changes in pressor responses to tyramine after equivalent doses of L-α-methyldopa.

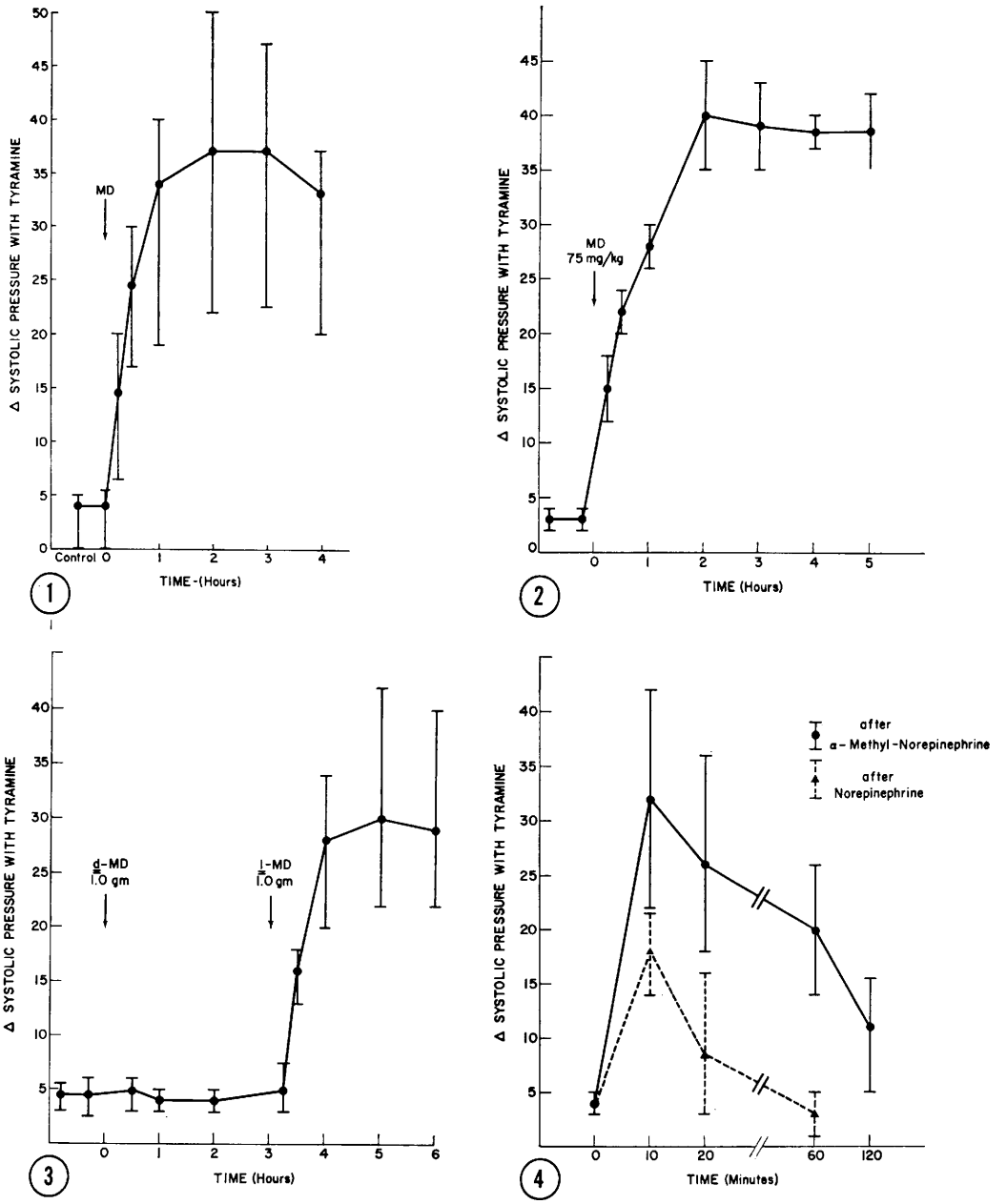

FIG. 1. Effects of a 1 g intravenous dose of methyldopa (MD) in enhancing pressor responses to repeated standard injections of tyramine in 7 dogs previously treated with reserpine. Mean systolic pressor increments and their ranges are shown for each time interval.

FIG. 2. Similar study to that in Fig. 1 showing effect of administration of 75 mg/kg of methyldopa on responses to tyramine in 4 reserpine-treated rabbits.

FIG. 3. Comparison of effects of 1 g intravenous doses of dextro (d) and levo (l) isomers of α-methyldopa on pressor responses to repeated injections of tyramine in 2 reserpine-treated dogs. The d-isomer was without effect.

FIG. 4. Comparison of enhancement of pressor responses to tyramine produced by 2 μg/kg doses of norepinephrine and α-methylnorepinephrine in the same 4 reserpine-pretreated rabbits.

TABLE I. Comparison of Effects of α-Methylnorepinephrine and Norepinephrine in Reserpine-Pretreated Rabbits and Dogs.

Δ Systolic blood pressure, mm Hg.

Animal	Direct pressor effects of test amines			Increases of pressor responses to tyramine after test amines		
	Dose (μg/kg)	L-α-methyl-norepinephrine	L-norepi-nephrine	Dose (μg/kg)	L-α-methylnor-epinephrine	L-norepi-nephrine
Rabbit 1	.5	45	104, 91	6	23	14, 12
" 2	.5	35	77, 93	6	33	15, 17
" 3	.5	58, 48	98	6	31, 35	21
" 4	.5	60, 42	94	6	42, 38	17
Dog 1	.1	54	64	2	42	26
" 2	.1	36	60	2	34	20

Comparison of D- *and* L-α-*methyldopa.* Experiments comparing the effects of equal doses of dextro and levo isomers of α-methyldopa were performed in 2 reserpine-treated dogs and revealed that the dextro-isomer (which is not metabolized to amines) differed from the levo-isomer in that it failed to restore pressor responses to tyramine (Fig. 3).

Effects of α-methyldopamine and α-methylnorepinephrine. The experiments were thereafter repeated in 2 reserpine-treated dogs and 2 reserpine-treated rabbits using the decarboxylation product of methyldopa, α-methyldopamine, in doses of 0.2 mg/kg and 2 mg/kg respectively. Restoration of tyramine activity similar to that found after intravenous doses of methyldopa occurred but peak effects developed slightly earlier, occurring within 45 to 60 minutes. In similar studies with α-methylnorepinephrine, the β-hydroxylated derivative of α-methyldopamine, immediate enhancement of responses to tyramine occurred even with small doses. Thus, 2 μg/kg of α-methylnorepinephrine in 4 rabbits and 6 μg/kg in 4 dogs produced pronounced increases in tyramine responses. In comparison to results after methyldopa, the enhancement of tyramine responses produced by the amine metabolites was of shorter duration and had disappeared within 4 hours after α-methyldopamine and within 3 hours after α-methylnorepinephrine.

Comparison of norepinephrine and α-methylnorepinephrine. It appeared of interest to compare the relative abilities of norepinephrine and α-methylnorepinephrine to raise blood pressure and to restore pressor effects of tyramine after reserpine. Both amines were therefore administered to each of 4 reserpine-pretreated rabbits and 2 pretreated dogs, varying the order of presentation of the amines and permitting sufficient time for complete disappearance of the effects of prior doses. It is noteworthy that, whereas norepinephrine had twice the pressor potency of α-methylnorepinephrine (Table I) its enhancement of pressor responses to tyramine was distinctly less and of shorter duration (Table I and Fig. 4).

Discussion. The present study demonstrates that methyldopa can temporarily restore pressor effects of tyramine in rabbits and dogs with reserpine-reduced sensitivity to tyramine, thus confirming our previous observations in man(6). Methyldopa itself, has been shown to be decarboxylated to α-methyldopamine in man and animals(10,11) and further conversion to α-methylnorepinephrine has been reported in the tissues of mice and rabbits(11). It appears that amine products mediate the effect of methyldopa on responses to tyramine since the D-isomer, which is not decarboxylated, is inactive and the amines α-methyldopamine and α-methylnorepinephrine also enhance tyramine responses. Further, the progressively shorter periods of onset with structures approaching that of α-methylnorepinephrine and the greater potency of the latter in this regard suggest it is the active form. These findings parallel the observations of other workers that α-methyldopa, α-methyldopamine and α-methylnorepinephrine restore pressor responses to tyramine in reserpine-treated cats(8,9) and increase the responses to sympathetic nerve stimulation of the isolated rabbit ileum and the cat nictitating membrane, after treatment with reserpine(9).

The aforementioned studies demonstrate pharmacologically that α-methyldopa and its amine metabolites enter peripheral sympathetic nerves, and provide an explanation for the preservation of tyramine responses in patients receiving methyldopa(6,7). It is noteworthy that other effects of methyldopa also appear to be dependent on its decarboxylation with formation of amine products. Thus, norepinephrine-depleting effects of methyldopa in guinea pigs(12) and hypotensive effects of methyldopa in renal hypertensive rats have been shown to be blocked by potent decarboxylase inhibitors(13).

Further insight into the mechanism of action of α-methyldopa has been provided by Muscholl and Maitre(14) who demonstrated that sympathetic nerve stimulation caused release of α-methylnorepinephrine from isolated hearts of α-methyldopa-treated rabbits thereby providing evidence that α-methylnorepinephrine may assume the role of a sympathetic mediator. It seems likely that differences in percent decarboxylation of the drug, in degree of displacement of norepinephrine by α-methylnorepinephrine, and in responsiveness to α-methylnorepinephrine as a false sympathetic mediator are all factors which contribute to species variations in hypotensive response, man being the most sensitive. The idea that blood pressure lowering might be due simply to substitution of a potent transmitter (norepinephrine) by a weaker one (α-methylnorepinephrine)(11) is complicated somewhat by the fact that α-methylnorepinephrine was more effective in the present studies than norepinephrine in restoring tyramine pressor reactions. Of course, only overall pressor responses were recorded and thus cardiac *versus* vascular contributions could not be determined. This, as well as the relative ability of norepinephrine and its α-methyl analogue to restore sympathetic nerve function in reserpinized preparations, warrant further study.

It should be kept in mind that with the exception of Day and Rand's report(9) that α-methyldopa produced impaired responses of the cat nictitating membrane to pre-ganglionic sympathetic nerve stimulation, it has not been possible to demonstrate defective sympathetic nerve function in animals following large doses of α-methyldopa(15,16). Also, it would be amiss still to ignore the possibility of a predominant site of action of α-methyldopa in the central nervous system.

Summary. Administration of methyldopa (L-α-methyldopa) to reserpine-treated dogs and rabbits results in restoration of pressor responses to tyramine. The amine metabolites of methyldopa, α-methyldopamine and α-methylnorepinephrine, cause similar but more prompt enhancement of tyramine responses with the latter compound showing maximum potency and more prompt onset of effect. That the effects of methyldopa are due to its amine metabolites is also indicated by the fact that D-α-methyldopa, which is not decarboxylated, is inactive. Present evidence favors α-methylnorepinephrine as the mediator responsible for the blood pressure lowering produced by methyldopa in man, as well as the preservation and restoration of tyramine responses following administration of the parent drug.

The α-methylated compounds used in these studies were supplied by Merck, Sharp and Dohme Laboratories, West Point, Pa.

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Mouse Leukocytes in Culture.* (30026)

H. G. WILLARD, I. B. H. HOPPE† AND P. NETTESHEIM‡ (Introduced by L. H. Smith)
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

In vitro culture of most mammalian cells has long been hampered by an inability to provide the proper conditions for maintenance of normal and functional activity. Recently, the technique developed by Moorhead *et al*(1) has allowed the continual maintenance of a sufficiently high metabolic state in human leukocytes so that mitosis, a normal cellular activity, could occur.

The culture of mouse leukocytes is especially appealing, since it has been shown(2, 3) that peripheral blood contains stem cells capable of promoting recovery of lethally irradiated rodents. Successful *in vitro* cultivation of these cells might lead to development of different stem cell lines devoted to formation of different, specific types of blood cells. Also, karyotype analysis of mouse leukocyte chromosomes without sacrifice of the donor would be of great advantage to mouse geneticists.

Leukocyte culture has been carried out with some success in a variety of animals, including the monkey(4); hamster(5); ox (6); snake(7); chicken(8); and rat(9). Nichols and Levan(10) have successfully applied this method to various laboratory mammals, including rat, guinea pig, rabbit, and mouse. Although human, rat, and guinea pig leukocytes have been successfully cultivated in our laboratory, and mitotic figures have been obtained from fresh mouse spleen and marrow, only sporadic success has been achieved with mouse peripheral leukocytes. The present study was undertaken to investigate culture conditions necessary for maintenance and growth of these cells.

Materials and methods. Male and female hybrid mice (C3H/Anf Cum × C57BL/Cum)F_1 were principally used in this study, although a few individuals of the BALB/c and C3H strains were also tested. Ages ranged from 2 weeks to 1½ years. Mice were narcotized with 1:10 nembutal or with ether, and were bled by cardiac or tail puncture into a syringe or tube rinsed with heparin or EDTA (1% in 0.7% NaCl). For some cultures, blood from individual mice was used; for others, the blood from a number of mice was pooled in a chilled tube.

Various agents were used for the separation of leukocytes. These included 3% dextran 1:1 (mol wt 275,000); 6% dextran (mol wt 75,000); 4% fibrinogen; and buffy coats obtained by centrifugation in a refrigerated centrifuge at speeds from 200-2000 rpm for 10-30 minutes. Heparinized whole blood was also tried. Separation at room temperature generally yielded a cleaner preparation of leukocytes.

A wide variety of culture conditions was used: maintenance of the cultures in a 5% CO_2 pH-regulated incubator; regulation of pH during culture from 5.8 to 7.6; varying erythrocyte concentration from 50,000 to 2.5 million per culture, and varying leukocyte concentration from 500,000 to 10 million per ml of culture medium; incubation with and without commercial Difco phytohaemagglutinin-M (PHA); use of a PHA prepared in our laboratory from cranberry beans; and

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† I. Medizinische Universitätsklinik, Berlin-Charlottenburg, Germany.

‡ Visiting investigator from Univ. of Freiburg, Germany.