Pharmacological Studies on Xanthine Derivatives

(II) ACTION ON PERIPHERAL VESSELS AND ACUTE TOXICITY

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The effect of 47 aminoxy and amino-xanthine derivatives on blood flow of the femoral artery (f. a.) and toxicity in mice and rats of some of these compounds were tested.

Comparing the effects of the compounds on f. a. and on coronary artery (c. a.), the former are more effective than the latter. The minimum effective dose of theophylline ethylenediamine (TE) and these compounds on f. a. is about 0.2 mg, and that on c. a. about 1.0 mg. But the potency indices of these compounds on f. a. are inclined to be smaller than on c. a. With regard to relationship between effect and chemical structure, although the effect on c. a. differs from that on f. a. in the little details, the correlation coefficient is 0.752, which seemed to indicate that both blood vessels react in a similar manner to the compounds.

The relationship between toxicity and chemical structure were studied with these interesting revelations: (1) In the monoalkylaminoxypyl xanthine derivatives, theobromine derivatives are more toxic than theophylline derivatives. (2) When C-atoms of the alkyl radicals replaced at amino radical increased, toxicity increased in direct proportion. (3) In the β -alkylaminoethyl theophylline derivatives, toxicity was strongest when the alkyl at terminal N was methyl, becoming less toxic in the case of ethyl and n-propyl in that order.

And the ratio of toxicity in intraperitoneal and intravenous injection were distributed from near 1.1 to near 6.8, indicating that the compounds are well absorbed.

IN THE PREVIOUS paper¹⁾, the coronary dilating effect of many newly synthesized xanthine derivatives were reported. In the present experiments, their dilating action on the peripheral vessels and their acute toxicity in mice and rats are investigated.

The coronary and peripheral vessels are distributed in tissues which are wholly different from each other in function. Whether or not their constriction and dilation are performed by the same mechanism is an interesting question. It is generally believed that the tension of the skin vessels and skeletal muscle vessels are maintained by the sympathetic nerve and dilated by the sympathetic cholinergic fiber^{2,3,4)}. On the coronary vessels

both the sympathetic and parasympathetic nerve are reported by many to have a dilating action^{5,6)}, while Katz et al⁷⁾ claimed that the sympathetic nerve maintains tension because coronary flow increased after this nerve was cut. According to Ikeda⁸⁾, stimulation of the sympathetic nerve resulted in transient constriction of the coronary vessels, followed immediately by dilatation. Further, the administration of epinephrine in a large dose is considered to act constrictively on the peripheral vessels dilatively in a small dose but, as claimed by some⁹⁾, constrictively even in a minimum effective dose. Others^{10,11)} claim that the vasodilative action of epinephrine is biphasic. Gottstein et al¹²⁾ reported that

epinephrine dilates the skeletal muscle vessels and constricts the skin vessels. On the coronary vessels, epinephrine has until recently been considered to have a dilating action, as coronary flow increased after it was administered. Now, however, a view was advanced by Allela et al¹³⁾ and by Eckstein¹⁴⁾ that the rate of myocardial oxygen consumption has a most important bearing on coronary flow. Hashimoto et al¹⁵⁾ reported that the principal effect of epinephrine is to constrict the coronary vessels and that any increase in coronary flow after its administration would be a secondary effect resulting from increased myocardial oxygen consumption. They admitted the presence of Ahlquist's¹⁶⁾ α -receptor in the coronary vessels, but denied the presence of β -receptor. From these observations, it is obvious that there is a considerable difference between the coronary and peripheral vessels.

In the present experiments, a femoral artery was used for a peripheral vessel and the action on blood flow in it was investigated. The action of each compound on it somewhat varied in intensity from that on the coronary vessels, indicating some responsive difference between the two types of vessels.

On the toxicities of alkylxanthine, oxyalkylxanthine and aminoalkylxanthine, a number of reports are already known^{17, 18, 19)} but few on those of the other xanthine compounds. In the present experiments, the LD₅₀ and minimum convulsant doses of the new xanthines in mice by intraperitoneal and intravenous injection were sought, as well as the convulsant dose in rats by intravenous infusion at a given rate. The results were found to have an interesting relation with their chemical structures.

METHODS AND TESTED COMPOUNDS

(a) Action on peripheral vessels.

Under anesthesia induced by intravenous injection of amobarbital sodium 40 mg/kg by the method of Cook et al²⁰⁾ a vinyl tube was connected to the femoral artery at a proximal and a distal point in the inguinal region of a dog weighing 10–20 kg, and blood flow through the tube was determined with an inserted flowmeter²¹⁾ in nearly the same manner as in the determination of coronary

blood flow²²⁾. To prevent coagulation, heparin sodium was given intravenously first in 300 u/kg and then in 100 u/kg at one hour intervals. As in the case of assaying coronary dilating action, the compounds to be tested were injected in 10 seconds in 0.2 cc of solution into the cannula between the flowmeter and the distal end. The solution was prepared by dissolving 2.5 mg of the compounds by hydrochloric acid in 0.2 cc of Ringer's solution. When it was acid or alkaline, the assay was made within the range in which the effect of pH was negligible. Change in blood flow was measured, as in the case of the coronary vessels, by computing the difference between blood flows in 3 minutes before and after administration of the compounds, and the results (potency index) were expressed as quotients of the test compound theophylline ethylenediamine (TE).

(b) Acute toxicity.

(i) LD₅₀ in mice

Normal mice weighing 15–20 g were treated intraperitoneally and intravenously and the LD₅₀ in 24 hours was sought. Computation was made by Van der Werden's method. The compounds used consisted of some of the compounds which were assayed for action on the coronary and peripheral vessels. When the toxicity was low, 50 mg/cc original solution was wholly given. Otherwise, the dose was 0.3 cc in intraperitoneal injection and 0.1 cc in intravenous injection. Each was administered in one minute.

(ii) Minimum convulsant dose (MCD)

The MCD in mice and rats was determined with some of the compounds which were assayed for LD₅₀. In mice, the compounds were given intravenously in one minute and the minimum dose that evoked convulsion was determined. The convulsion process was followed macroscopically. The convulsion inducing dose (CID) in rats was the total amount that was infused into the vein (0.05 cc/10 sec, 25 mg/cc solution), at a constant rate until convulsion occurred.

(c) Tested compounds.

The majority of the compounds described in the previous report¹⁾ were assayed for peripheral action. Table I gives their code designations, together with their potency index. The LD₅₀ of some of the compounds are shown in Tables 2 and 3.

RESULTS

(a) Peripheral action

The tested compounds include 47 aminoxyalkyl and aminoalkyl derivatives (subgroups

A, B-I, II, III, IV of groups 1, 2 in the previous report) which were assayed for coronary dilating action. TE, which was used as the standard, acts more strongly on the peripheral vessels than on the coronary vessels, and while the minimum effective dose for the latter is about 1.0 mg, 0.3–0.25 mg is sufficient for the former. Most of the tested compounds were generally weaker on the peripheral vessels.

Compounds with potency index of above 1.30 numbered three and those over 1.0 totaled eleven.

Fig. 1 records on smoked paper the increased peripheral blood flow after administra-

Fig. 1. Effect of intraarterial injected acetylcholine, epinephrine, TH-60 and theophylline ethylenediamine on femoral blood flow and systemic blood pressure in dogs. Figure represents from top to bottom; blood pressure, femoral blood flow and time in minutes.

tion of 0.5 mg of TE, 0.5 mg of TH-60, 1 μ g epinephrine, and 1 μ g of acetylcholine (ACh). It will be seen in this figure that constriction by epinephrine was followed by dilatation. Only dilatation followed the administration of ACh, TH-60 and TE. Administration of TH-60 produced a slight change in blood pressure. The relation of peripheral and coronary action of the compounds to their chemical structures is shown in Figs. 2–6. Fig. 2 compares 1-derivatives of theobromine (group A) and 7-derivatives of theophylline

Fig. 3. Relationship between chemical structure and effect on femoral and coronary blood flow. Comparison between secondary amines and tertiary amines in 7-substituted theophylline derivatives. (See Fig. 2 and text)

sels. When they had n-propyl or n-butyl, the tertiary amine derivatives were stronger to a very large degree. This was the same as with the coronary vessels.

Viewed from the number of carbon atoms, the secondary amine derivatives were stronger in the case of one C-atom methyl; the secondary and tertiary amines had nearly the same potency in the case of 2 C-atoms; while the tertiary amines inversely became stronger when the number of C-atoms was 3 or more.

Fig. 4 shows the peripheral action of the tertiary amines of subgroup B-I that possess methyl, ethyl, n-propyl, n-butyl, n-amyl, i-propyl, i-butyl and i-amyl both as R_3 and

R_4 . When the alkyl was normal, the methyl derivatives were stronger than the ethyl derivatives. But in general, the action became stronger as the number of C-atoms increase, the n-amyl derivatives being stronger than the n-butyl-derivatives. Also in the iso-derivatives, the action was stronger the higher the number of C-atoms, the highest potency

Fig. 4. Relationship between chemical structure and effect on femoral and coronary blood flow. Comparison between the effect of each alkyl compounds in tertiary amines. (See Fig. 2 and text)

being shown by the i-amyl-derivatives. This was in contrast to the cases of coronary action, in which the n-propyl derivatives showed the highest potency, followed to a slightly weaker degree by the i-amyl, n-amyl, and n-butyl, and i-butyl derivatives. Especially prominent was the difference in the n-propyl derivatives, where peripheral action was nearly half as low as TE. Further, in the case of the propyl and butyl derivatives, the i-compounds weaker than the n-compounds, showing about the same potency as those having one less C-atom. In the case of the amyl-derivatives, on the other hand, the i-compounds showed far stronger action. Fig. 5 compares subgroups B-I, III and IV in cases when R_3 and R_4 were both methyl (in I and III), ethyl, n-propyl, n-butyl (in I and III) and i-butyl. First, the

TABLE I

(1) AMINOXYALKYL-XANTHINE DERIVATIVES
 SUBGROUP A: Theobromine derivatives
 A-1 β -OH derivatives

No. of Comp.	Name	Potency Index	No. of Comp.	Name	Potency Index
TH-26	1 (γ -methylamino β -oxypropyl) theobromine	0	TH-11	1 (γ -piperidino β -oxypropyl) theobromine	0.25
TH-25	1 (γ -ethylamino β -oxypropyl) theobromine	0.05	TH-10	1 (γ -morpholino β -oxypropyl) theobromine	0.17
TH-27	1 (γ -n-propylamino β -oxypropyl) theobromine	0.12	TH-34	1 (γ -pyrrolidinyl β -oxypropyl) theobromine	0.37
TH-33	1 (γ -i-propylamino β -oxypropyl) theobromine	0.07	TH-36	1 (γ -phenethylamino β -oxypropyl) theobromine	0.64
TH-28	1 (γ -n-butylamino β -oxypropyl) theobromine	0.02			

SUBGROUP B: Theophylline derivatives
 B-1 β -OH derivatives

TH-5	7 (γ -methylamino β -oxypropyl) theophylline	0.19	TH-62	7 (γ -di n-amylamino β -oxypropyl) theophylline	1.37
TH-1	7 (γ -dimethylamino β -oxypropyl) theophylline	0.15	TH-54	7 (γ -di i-amylamino β -oxypropyl) theophylline	1.83
TH-13	7 (γ -ethylamino β -oxypropyl) theophylline	0.05	TH-15	7 (γ -allylamino β -oxypropyl) theophylline	0.31
TH-4	7 (γ -diethylamino β -oxypropyl) theophylline	0.07	TH-43	7 (γ -diallylamino β -oxypropyl) theophylline	0.20
TH-14	7 (γ -n-propylamino β -oxypropyl) theophylline	0.05	TH-2	7 (γ -piperidino β -oxypropyl) theophylline	0
TH-29	7 (γ -di n-propylamino β -oxypropyl) theophylline	0.47	TH-3	7 (γ -morpholino β -oxypropyl) theophylline	0.17
TH-30	7 (γ -di i-propylamino β -oxypropyl) theophylline	0.17	TH-17	3 (γ -pyrrolidino β -oxypropyl) theophylline	0.22
TH-16	7 (γ -n-butylamino β -oxypropyl) theophylline	0.22	TH-37	7 (γ -phenethylamino β -oxypropyl) theophylline	0.53
TH-31	7 (γ -di n-butylamino β -oxypropyl) theophylline	1.20	TH-24(0)	7 (γ -di i-butylamino β -oxypropyl) theophylline	0.61

B-2 β -esterified derivatives

TH-6	7 (γ -dimethylamino β -benzoyloxypropyl) theophylline	0.54	TH-53	7 (γ -di n-butylamino β -acetoxypropyl) theophylline	1.27
TH-7	7 (γ -dimethylamino β -acetoxypropyl) theophylline	0.54	TH-24(1)	7 (γ -di i-butylamino β -benzoyloxypropyl) theophylline	1.05
TH-38	7 (γ -diethylamino β -benzoyloxypropyl) theophylline	0.63	TH-24(2)	7 (γ -di i-butylamino β -acetoxypropyl) theophylline	0.92
TH-20	7 (γ -di n-propylamino β -benzoyloxypropyl) theophylline	1.22	TH-39	7 (γ -morpholino β -benzoyloxypropyl) theophylline	0.41
TH-58	7 (γ -di n-propylamino β -acetoxypropyl) theophylline	1.02	TH-40	7 (γ -piperidino β -benzoyloxypropyl) theophylline	1.15
TH-23	7 (γ -di n-butylamino β -benzoyloxypropyl) theophylline	1.56	TH-41	7 (γ -pyrrolidinyl β -benzoyloxypropyl) theophylline	1.27

(2) AMINOALKYL-XANTHINE DERIVATIVES
 B-3 aminopropyl derivatives

No. of Comp.	Name	Potency Index	No. of Comp.	Name	Potency Index
TH-50	7 (γ -diethylaminopropyl) theophylline	0.29	TH-52	7 (γ -di i-butylaminopropyl) theophylline	0.92
TH-51	7 (γ -di n-propylaminopropyl) theophylline	0.88			

B-4 aminoethyl derivatives					
<i>TH-57</i>	7 (β - <i>dimethylaminoethyl</i>) <i>theophylline</i>	0.83	<i>TH-60</i>	7 (β - <i>di n-butylaminoethyl</i>) <i>theophylline</i>	1.10
<i>TH-63</i>	7 (β - <i>diethylaminoethyl</i>) <i>theophylline</i>	0.31	<i>TH-59</i>	7 (β - <i>di i-butylaminoethyl</i>) <i>theophylline</i>	0.54
<i>TH-61</i>	7 (β - <i>di n-propylaminoethyl</i>) <i>theophylline</i>	0.75

Fig. 5. Relationship between chemical structure and effect on femoral and coronary blood flow. Comparison between aminoethyl theophylline derivatives and aminopropyl theophylline derivatives with OH and others without OH at β . (See Fig. 2 and text)

theophylline derivatives with an ethylene bridge between 7-N and amino-N were compared with those with a propylene bridge, and hardly any difference was found between them when they were γ -diethylamino derivatives. But when they were *n*-propyl and *i*-butyl derivatives, the propylene bridge compounds were stronger. In coronary dilating action the propylene bridge compounds were weaker than the ethylene bridge compounds. Next, comparing the propylene bridge compounds with OH at β position and those without it, the OH-deficient compound were found to be stronger. This was also different from the

case of the coronary vessels. The ethylene-bridge compounds on the whole showed a stronger action, varying less than the other 2 groups with the change in the number of C-atoms, while the methyl derivatives possessed a fairly strong action nearly comparable to the *n*-butyl derivatives. The tendency for the ethyl derivatives to be weaker than the methyl derivatives was also present in the propylene-bridge compounds with β -OH. Comparing the ethylene compounds and propylene compounds with β -OH, the former were stronger as long as the number of C-atoms was low. But as the number of C-atoms increased in the methyl, ethyl and *n*-propyl radical in that order, the difference between them grew less, and the β -OH-

Fig. 6. Relationship between chemical structure and effect on femoral and coronary blood flow. Comparison between aminopropyl theophylline derivatives with OH and others with OH esterified at β . (See Fig. 2 and text)

propylene group inversely became stronger when they had the *n*- and *i*-butyl radical. Fig. 6 compares the effects of esterification in those of groups B-I and -II in which both R_3 and R_4 were methyl, ethyl, *n*-butyl and *i*-butyl, and those which contained the cyclic amines of pyrrolidino, morpholino, and piperidiny. In all the cases, esterification enhanced the action. This was the same as in the case of coronary action.

In this way, there were differences in the little details between coronary and peripheral action. But none of the compounds acted exclusively on one of the two kinds of vessels but not on the other, and none induced opposite responses in them. The correlation coefficient between coronary and peripheral action was 0.752, which is significantly high.

(b) *Acute toxicity*

(i) LD_{50} in intraperitoneal injection

After the administration of these chemicals, many mice died of asphyxiation resulting from clonic or tonic convulsion. But some of very slight toxicity caused no convulsion, and death seemed to be attributed to other causes. Thus, there is obviously a distinct qualitative difference in the toxicity of the compounds. Figs. 7-11 represent the relationship between chemical structures and quantitative differences in toxicity, without regard to qualitative differences. Fig. 7 shows LD_{50} of the majority of the compounds in Fig. 2. In the case of the *n*-propyl-derivatives, the theobromine group showed a lower toxicity, but

Fig. 7. Relationship between chemical structure and LD_{50} in mice. Comparison between 1-substituted theobromine derivatives and 7-substituted theophylline derivatives. Vertical axis indicates LD_{50} in mg/kg. Horizontal axis indicates numbers of compounds and substitution groups at R_3 . (See text)

in the others, the theophylline group was always weaker. This is different from what is generally believed. The methyl-derivatives had a stronger toxicity than the ethyl-derivatives both in the theophylline and the theobromine group. But their toxicities were always lower than that of theophylline itself—as low as 1/5 to 1/8. Fig. 8 shows the toxicities of the majority of the compounds in Fig. 3. In the case of the methyl derivatives, the tertiary amine group had a lower toxicity; but in the case of the alkyl deriva-

TABLE II

No. of Comp.	LD_{50} (95% fiducial limit) mg/kg	No. of Comp.	LD_{50} (95% fiducial limit) mg/kg	No. of Comp.	LD_{50} (95% fiducial limit) mg/kg
TH-26	1093 (1053-1134)	TH-30	828.1 (795.4-864.2)	TH-53	123.5 (115.6-132.1)
TH-25	1852 (1784-1922)	TH-16	1141 (1118-1165)	TH-24(1)	1835 (1763-1960)
TH-27	1581 (1472-1698)	TH-31	128.3 (119.5-137.8)	TH-24(2)	491.0 (453.1-533.5)
TH-28	1036 (984.5-1090)	TH-24(0)	1095 (1028-1169)	TH-50	729.6 (676.1-794.3)
TH-34	1654 (1526-1793)	TH-62	114.1 (104.6-124.6)	TH-51	269.0 (256.7-281.5)
TH-5	1585 (1494-1684)	TH-54	103.9 (97.2-111.1)	TH-52	220.9 (190.6-256.1)
TH-1	1772 (1605-1956)	TH-17	2067 (1966-2176)	TH-57	124.6 (112.1-138.6)
TH-13	1933 (1885-1983)	TH-37	243.6 (213.6-277.7)	TH-63	223.1 (218.0-228.3)
TH-4	1212 (1165-1261)	TH-20	307.4 (297.7-317.5)	TH-61	334.0 (311.6-357.9)
TH-14	1355 (1332-1377)	TH-58	328.4 (307.2-351.1)	TH-60	157.7 (142.6-174.6)
TH-29	279.5 (246.2-317.3)	TH-23	125.9 (113.0-140.3)	TH-59	1338 (1273-1408)
TH-49	2224 (2115-2351)

Fig. 8. Relationship between chemical structure and LD₅₀ in mice. Comparison between secondary amines and tertiary amines in 7-substituted theophylline derivatives. Horizontal axis indicates numbers of compounds and substitution groups at R₃ and R₄. (See text)

tives higher than the ethyl derivatives, the tertiary amine group gradually gained in toxicity; and in the case of the n-butyl derivatives, the toxic ratio of the tertiary to the secondary amine group was 9:1. Fig. 9 compares the toxicities of the compounds in Fig. 4. In the n-compounds, toxicity rose sharply as the number of C-atoms increase. Namely, the n-propyl derivatives were slightly weaker, the n-butyl derivatives rather stronger, and the n-amyl derivatives unmistakably stronger than TE. In the i-compounds, the i-propyl derivatives were far weaker than the n-propyl derivatives. The i-butyl derivatives were even weaker than the i-propyl derivatives, almost equaling the ethyl derivatives; but the i-amyl derivatives were nearly as strong as the n-amyl derivatives. Fig. 10 compares the toxicities of the compounds in Fig. 5. The ethylene bridge compounds differed greatly from both groups of the propylene bridge compounds in that the methyl-derivatives had

Fig. 9. Relationship between chemical structure and LD₅₀ in mice. Comparison between the effect of each alkyl compounds in tertiary amines. (See Fig. 8 and text)

Fig. 10. Relationship between chemical structure and LD₅₀ in mice. Comparison between the aminoethyl theophylline derivatives and aminopropyl theophylline derivatives with OH and others without OH at β. (See Fig. 8 and text)

the highest toxicity; that the higher the number of C-atoms, the more toxicity lessened in the ethyl and n-propyl derivatives in that order; but that the n-butyl derivatives again neared the methyl-derivatives in toxic effect. But the i-butyl derivatives were the weakest of the 5 compounds of the ethylene bridge group. Among the propylene compounds, all having β-OH were weaker than the β-OH-deficient

ones which retained their toxic effect even in the case of the *i*-butyl derivatives. Fig. 11 shows the toxicities of the compounds in Fig. 6. The *n*-propyl derivatives were weakened by esterification, whereas the *n*-butyl derivatives had their toxicity increased by the same

Fig. 11. Relationship between chemical structure and LD₅₀ in mice. Comparison between aminopropyl theophylline derivatives with OH and others with OH esterified at β . Numbers over each histogram indicate the LD₅₀ in intraperitoneal injection and numbers in parentheses indicate the LD₅₀ in intravenous injection.

process, though slightly. However, the benzoyl esters of the *i*-butyl derivatives showed a weakened toxicity, while the acetyl esters of same, on the other hand, had an increased toxicity. But in all cases, the toxicities of the esterified compounds remained near those of the mother compounds and never underwent so radical a change as to warrant being called inversion; the LD₅₀ of the β -esterified *n*-propyl derivatives remained near 300 mg/kg and that of the β -esterified *n*-butyl derivatives near 125 mg/kg. Esterification of the *i*-butyl derivatives increased toxicity to 491 mg/kg in the case of acetyler and decreased it to 1835 mg/kg in the case of benzoyler, but they could not equal the *n*-butyl and *n*-propyl derivatives.

(ii) LD₅₀ in intravenous injection.

Compounds with code designations of TH-29, -20, -58, -31, -23, -24(0), 24(1) and 24(2) were assayed for acute toxicity by intravenous administration in mice. All of them induced clonic convulsion, leading to death in several minutes to one hour. The animals which survived this period did not die within 24 hours.

Table III contains the ratios of intravenous LD₅₀ to intraperitoneal LD₅₀ (P/V). TH-24(0), -24(1), and -24(2), which had a very low toxicity in intraperitoneal injection, were highly toxic in intravenous injection, having an LD₅₀ of 233.5, 272.7 and 190.6 mg/kg respectively. This order of toxicity was the same as in intraperitoneal injection

TABLE III

No. of Comp.	LD ₅₀ (i.p.) mg/kg	LD ₅₀ (i.v.) (95% fiducial limit) mg/kg	P/V Ratio	MCD mg/kg	CID mg/kg	CID/MCD
TH-29	279.5	162.8 (153.4-170.6)	1.71	12.5	75	6.0
TH-20	307.4	146.8 (140.7-153.6)	2.15	12.0	—	—
TH-58	328.4	155.5 (150.4-160.6)	2.23	17.5	—	—
TH-51	269.0	—	—	22.5	—	—
TH-61	334.0	134.8 (122.4-146.3)	2.47	17.5	—	—
TH-31	128.3	113.3 (100.5-122.8)	1.17	1.5	42.5	28.0
TH-23	125.9	109.4 (102.1-115.5)	1.22	3.5	30.0	8.5
TH-53	123.5	98.2 (91.0-106.1)	1.18	2.5	—	—
TH-60	157.7	75.5 (70.3-81.5)	2.10	3.5	—	—
TH-24(0)	1095	233.5 (223.4-243.6)	4.70	8.0	47.5	6.0
TH-24(1)	1835	272.7 (264.9-280.8)	6.84	35.0	125.0	3.5
TH-24(2)	491.0	190.6 (183.6-198.0)	2.62	6.5	42.5	6.5
TH-52	220.9	140.3 (128.2-151.1)	1.57	8.0	—	—
TH-59	1338	—	—	35.0	—	—

(See Fig. 11). The P/V ratios were 4.7, 6.8 and 2.6 respectively. Of the *n*-propyl derivatives (TH-29, -20 and -58), the last two had P/V ratios of 2.15 and 2.23. The P/V ratios of the *n*-butyl derivatives (TH-31, -23 and -53) were very small (1.17, 1.22 and 1.18 respectively). But in all the cases, the order of LD₅₀ was the same in both intravenous and intraperitoneal administration.

(iii) MCD in intravenous administration in mice.

The MCD of TH-29, -20, -58, -51, and -61, which have an *n*-propyl radical at the end, ranged between 12 mg/kg and 22 mg/kg, and that of TH-31, -23, -53 and -60, which have an *n*-butyl radical, ranged between 1.5 mg/kg and 3.5 mg/kg, 6–8 times as potent as the former. In contrast, TH-24(0), -24(1), -24(2), -52 and -59 having an *i*-butyl radical were different, for while TH-24(1) and -59 were far less toxic than the *n*-propyl group with an MCD of 35 mg/kg, the others were more toxic with 6.5 mg/kg–9.5 mg/kg.

(iv) Convulsion inducing doses (CID) under constant intravenous infusion in rats.

The ratios of minimum convulsant dose to convulsion inducing dose (MCD/CID) are appended to Table III. The CID of TH-29 of the *n*-propyl derivatives was 75 mg/kg, which is 6 times as great as the MCD in mice. As for the *n*-butyl derivatives, the CID of TH-23 was 8.5 times, and that of TH-31 28 times as great as the MCD in mice. As for the *i*-butyl derivatives, the CID of TH-24(0) was 6 times, that of TH-24(1) 3.5 times, and that of TH-24(2) 6.5 times as toxic as their MCD in mice. In this way, the CID in rats was 3.5–6.5 times greater than the MCD in mice, with the exception of the *n*-butyl derivatives.

DISCUSSION

(a) In 1911, Sollman²³⁾, after detailed experiments, reported the vasodilative action of xanthine on the peripheral vessels, which up to that time had been subject to disagreement, and since then the action of this compound

has been considered an established fact. Subsequently, this aroused the speculation that xanthine also exerts a dilative action on the coronary vessels, and when it was confirmed by many researchers to increase coronary flow, the first step in the treatment of coronary disease was taken. A few researchers^{24,25)} experimentally demonstrated the vasodilative action of xanthine on the extremities, but this is not yet sufficiently recognized as to be considered for clinical application. Very recently Armitage et al reported on the extremely close correlation between coronary and peripheral action of 6-thioxanthine derivative²⁶⁾.

In the present experiments, it was mainly investigated what responses the peripheral and coronary vessels display to the xanthine derivatives. At the same time the relation between chemical structure and action was sought.

Action of the xanthine derivatives on the peripheral vessels was different from coronary action in the following points: (1) The theobromine derivatives are inclined to be more potent than the theophylline derivatives when they are monoalkylamino β -oxypropyl xanthine derivatives. (2) The derivatives with secondary amines at position γ are stronger than those with tertiary amines when the replaced alkyl radicals are methyl. (3) Potency, which rises with the increase in the number of C-atoms of the replaced alkyl radical, attains a maximum at 4 (butyl-derivatives) on the coronary vessels, whereas on the peripheral vessels it is higher at 5 (amyl derivatives). (4) The propylene-bridge theophylline derivatives (between N at position 7 and N of the amine) are stronger than the ethylene-bridge derivatives. (5) The propylene-bridge theophylline derivatives which lack β -OH are stronger than those which have it. (6) In the cases of the propylene-bridge compounds having β -OH and the ethylene bridge compounds, the γ -dimethyl derivatives are stronger than the γ -diethyl derivatives.

In this way, the peripheral action differs from the coronary action, suggesting a difference in response to the compounds between the two kinds of vessels. But, nevertheless, the coefficient of 0.752 points to a high correlation between the

action intensities on them. The fact that no compounds effected only one of them but not the other and none acted inversely to each other on them would indicate the possibility that both kinds of vessels have an extremely similar character at the site of action on these xanthine derivatives. And the fact that these xanthine derivatives have no stimulative action on myocardium and do not increase oxygen consumption of the muscle excessively²⁷⁾ would indicate that the method used in the previous experiments of measuring the coronary blood flow is best for showing the coronary dilating action of these compounds.

TE has a considerably potent dilative action on the peripheral vessels and the comparative effects of the tested compounds on the basis of TE are lower on the peripheral than on the coronary vessels. The minimum effective dose of TE and the tested compounds on the peripheral vessels was about 0.2 mg/10 sec/0.2 cc, which indicates that the peripheral vessels respond more keenly the coronary vessels. This seems to contradict the clinical observation²⁴⁾ that the dilative action of xanthine derivatives on the peripheral vessels is very transient. Assumedly, the peripheral vasodilative action is masked by the central vasoconstrictive action²⁵⁾ of the compounds.

(b). Toxicity

The theobromine derivatives would naturally be expected to have a weaker toxicity than the theophylline derivatives, since the mother substance of the former is weaker than that of the latter. In the present experiments, however, the theobromine group were more toxic than the theophylline group except the *n*-propyl derivatives (see Fig. 7). The same results are already known concerning some of the alkyl-xanthines. McColl et al¹⁷⁾ reported that when 1-allyl and 1-butyl theobromine were compared respectively with 7-allyl and 7-butyl theophylline, the theobromine derivatives were more toxic in both cases. But 7-*i*-amyl theophylline does not differ from 1-*i*-amyl theobromine in toxicity. On the other hand, there are many theophylline deri-

vatives which have a higher toxicity than the corresponding theobromine compounds. Kuschinsky²⁸⁾ compared ST (7-diethylaminoethyl theophylline), MT (7 (β -hydroxypropyl) theophylline) and OT (7 (β -oxyethyl) theophylline) with STB (1-diethylaminoethyl theobromine), MTB (1 (β -oxypropyl) theobromine) and OTB (1 (β -oxyethyl) theobromine) respectively, and Giertz¹⁹⁾ compared OT with OTB. Both reported the theobromine derivatives to be weaker in toxicity.

Koch²⁹⁾ and Dawake³⁰⁾ performed experiments on TH-63, one of the tested compounds in the present experiments and which was referred to as ST in theirs, and gave 182 mg/kg as its LD₅₀ in mice. This is in disagreement with 223 mg/kg obtained by the author in the present experiments. For theophylline, however, Koch and Dawake gave considerably different figures of 184 mg/kg and 215 mg/kg respectively, while in the present experiments the LD₅₀ of theophylline was 266.8 mg/kg. In the present experiments, the toxicity of theophylline and other xanthine derivatives were generally lower than the results reported by others, but there were no differences in the order of toxicity.

As shown in Fig. 8, toxicity ascends in the order of TH-13, -1, -5, -14, -4, -16, -29, -31. On the other hand, the number of C-atoms in the alkyl radical introduced to 7-N increases in the order of TH-5, -1, -13, -14, -4, -16, -29, -31. It is interesting to note that those two orders are in close conformity, except for the TH-5 having an *N*-methyl.

In Fig. 10 it is interesting to see that in theophylline derivatives which have an ethylene bridge between 7-N and amino-N at the end, an increase in the number of C-atoms in the alkyl radical introduced into the amino radical does not mean an increase in toxicity, which is maximal in the case of the methyl radical, decreasing with the increase of C-atoms and again becoming high in the *n*-butyl derivative. It is also noteworthy in this group that the *i*-butyl derivatives are very weak, their toxicity being below 1/4 that of the *n*-alkyl derivatives. In the pro-

pylene bridge group without β -OH, the *i*-butyl derivatives do not show a decline in toxicity, indicating there is agreement between the increase of C-atoms and increase in toxicity.

There is a wide difference in toxicity between the *i*-butyl derivatives (TH-24(0), -24(1) and -24(2)) and the *n*-butyl derivatives TH-31, -23 and -53). Difference in absorption may account for this.

This can be confirmed by observing the relation between intraperitoneal and intravenous toxicity. The P/V ratios for the *n*-butyl derivatives are 1.17, 1.22 and 1.18, indicating that they can be absorbed in intraperitoneal as well as in intravenous injection. Against this, the ratios for the *i*-butyl derivatives are 4.7, 2.62 and 6.84, indicating slow absorption in intraperitoneal injection. These are, however, by no means extraordinary for this series of compounds, since the ratios for theophylline and theobromine are about 3. At any rate, intraperitoneal and intravenous toxicity difference in toxicity due to difference in absorption need not be taken into much consideration in these compounds.

As to change in toxicity caused by esterification, the *n*-propyl derivatives showed less toxicity in intraperitoneal injection but greater toxicity in intravenous injection. It is believed that esterification increased toxicity, but slow absorption from peritoneum diminished it. The toxicity of the *n*-butyl derivatives after esterification was seen to increase in both injections, so that the question of absorption was in no way involved. Further, in the *i*-butyl derivatives, the LD_{50} in both intraperitoneal and intravenous injections were diminished in the benzoyl esters and increased in the acetyl esters. In this case, too, absorption had no bearing on toxicity, the results of intraperitoneal injection reflecting toxicity fairly reliably.

The ratios of intravenous LD_{50} to the MCD were very large at 73.9, 29.4, 41.8 and 22.8 respectively for TH-31, -23, -53 and -60, all of which have the *n*-butyl radical. TH-24(0), -24(1), -24(2) and -52, which have the *i*-butyl radical, gave smaller values of 29.2, 7.7, 28.8 and 17.5 respectively.

TH-29, -20, -58 and -61, which have the *n*-propyl radical, gave still slightly smaller values of 22.4, 11.9, 8.4 and 7.7. These figures indicate that the *n*-butyl derivatives can induce convulsion in a very small dose ($1/20$ – $1/70$ of LD_{50}) but that the other two groups require $1/7$ – $1/30$. Namely, the first group can easily evoke convulsion.

The convulsion inducing dose in rats in constant intravenous infusion nearly parallels the MCD in mice, except only for TH-23 and -31 which have approximately the same CID as those of the others in spite of a high toxicity in the MCD-test. Presumably, the rate of transfer from blood circulation to the central nervous system accounts for this, but elucidation must await further studies.

SUMMARY AND CONCLUSION

Newly synthesized xanthine derivatives were assayed for peripheral vascular action and toxicity. The peripheral action of some of them differed in intensity from their coronary action which was reported in the previous paper. But few manifested a totally different trend on the two kinds of vessels, and the correlation coefficient of 0.752 indicates that coronary and peripheral action are approximately identical. But when expressed as a quotient of TE, the peripheral action of most of the compounds which were tested in the present experiment were weaker than that of theophylline. Prominent differences between the two actions are:

- 1) Of the tested compounds, those which showed an especially marked coronary-peripheral difference were TH-29, -24(0), -30 and -57. The peripheral action of the first 3 were as low as $1/3$, $1/2$ and $1/5$ of their coronary action respectively, while the peripheral action of TH-57 was about 3 times as high as its coronary action.
- 2) The peripheral action of the γ -amino β -oxypropyl derivatives intensified as the number of C-atoms increase in the alkyl radical introduced into the γ -amino radical. But unlike in the case of coronary action, it was highest not at C_4 but at C_5 .
- 3) When the theophylline derivatives with

a propylene-bridge between 7-N and terminal N and having β -OH were compared with those without β -OH, the latter group showed a stronger action on the peripheral vessels, whereas it was weaker in coronary action.

4) Of the ethylene-bridge compounds, those having the ethyl, n-propyl, n-butyl and i-butyl radical at γ -N, were nearly equal in coronary and peripheral action, whereas TH-57 having a methyl radical displayed a much stronger peripheral action.

Further, an interesting relation was revealed between intraperitoneal LD₅₀ in mice and chemical structure:

1) When five γ -monoalkylamino- β -oxy-propyl theobromines were compared with the corresponding 5 theophylline compounds, the formers had a stronger toxicity, with the sole exception of the n-propyl derivative.

2) In the β -oxy-propylene bridge theophyllines which have normal alkyls at position γ , toxicity increased in direct proportion to the increase in the total number of C-atoms in the alkyl radicals, notwithstanding the secondary or tertiary amines.

3) In the theophylline group which has an ethylene bridge between 7-N and terminal N, toxicity was strongest when the alkyl at terminal N was methyl, becoming less toxic in the case of ethyl and n-propyl in that order, again becoming strong in the case of n-butyl, but being very weak in the case of i-butyl.

4) In the propylene-bridge compounds deficient of β -OH, toxicity did not diminish when the γ -alkyl was i-butyl.

As for the relation between intraperitoneal and intravenous toxicity:

1) The P/V ratios were distributed from near 1.1 to near 6.8, and this fact indicates that these compounds have well been absorbed by intraperitoneal injection.

2) Also from the minimum convulsion dose it was seen that TH-31, -23, -53 and -60, which have an n-butyl radical, possess a very strong toxicity, while TH-24-(1) and -59, which have an i-butyl, are rather low in toxicity.

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