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Epinephrine-induced Hyperuricemia in Experimental Animals[1)]

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l-Epinephrine administered intraperitoneally in rats caused a hyperuricemia, which can be inhibited by the pretreatments with *alpha*-adrenergic blockers, but not with *beta*-adrenergic blockers. This was also inhibited by the pretreatments with ouabain, adrenochrome and allopurinol. The mechanism of the epinephrine-induced hyperuricemia was, therefore, considered to be due to the stimulation of ATP-degradation by epinephrine *via alpha*-adrenergic receptor.

Keywords——hyperuricemia; α-action of catecholamine; *l*-epinephrine; uric acid; xanthine oxidase; adrenergic blocking agents; metabolic inhibitors; rat; dog

In 1957, Imaizumi, *et al.*[3)] proposed that catecholamines have hypertension and hyperuricemia inducing actions. The effects reported by them, however, were not sufficiently marked as to suggest any clinical interest, and they have not subsequently been investigated in detail. On the other hand, several biological studies[4,5)] have suggested that the rate-limiting process of purine metabolism is involved in the first step of *de novo* and salvage synthesis of nucleotides, and this has stimulated much interest in the physiology of the degradative pathway of purine compounds. However, our previous investigation[6)] has shown that the purine degradative process is affected by the administration of steroid hormones and by the removal of hormone producing organs, and this suggests that the process is not simply a catabolic pipe of purine compounds, but itself able to be controlled by steroid hormones. In these investigations, the hormonal effects to the level of plasma uric acid were dependent upon the intact level in rats, indicating the presence of a more direct factor than steroid hormones in the control of purine catabolism.

Another important observation was that the plasma level of uric acid is several fold higher than the intact level under ether anesthesia, but not under pentobarbital anesthesia, and this suggested that epinephrine from the adrenal medulla stimulates the elevation of plasma uric acid.

The present investigation has confirmed the action of catecholamines on uric acid level, and the mode of action of epinephrine in inducing hyperuricemia is discussed here in terms of adrenergic receptor stimulation.

Materials and Methods

Animals——Male Wistar strain rats in their seventh week were used for most of the experiments. They were maintained on solid food and water *ad libitum*.

Agents——1-Benzyl-2-(3-methylisoxazol-5-yl)carbonylhydrazine, prepared in our laboratory, is a monoamine oxidase inhibitor.[7)] Other agents used are commercial preparations. These agents were injected intraperitoneally.

1) This work was presented in part at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April, 1972.
2) Location: *Fukushima-ku, Osaka, 553, Japan.*
3) R. Imaizumi, H. Yagi, Z. Shibuya, H. Matsumoto, Y. Watari, K. Sano, and K. Nakajima, *Shinryo* (in Japanese), **10**, 510 (1957).
4) S. Morisawa and M. Yukioka, *Taisha* (in Japanese), **5**, 168 (1968).
5) W.N. Kelley, W. Grobner, and E. Holmes, *Metabolism*, **22**, 939 (1973).
6) Y. Ogawa, Y. Yonetani, and K. Takano, *Jap. J. Pharmacol.*, in preparation.
7) A. Kurosawa, *Chem. Pharm. Bull.* (Tokyo), **17**, 36 (1969).

Analysis——Plasma uric acid was determined by autoanalyzer.[8] Xanthine oxidase activity in liver extract was determined by Kalcker's method.[9] The liver extract was prepared as follows: Liver was removed, weighed and homogenized with Krebs–Ringer bicarbonate buffer to obtain 20% homogenate. The homogenate was centrifuged for 10 min at 25000×*g* and recentrifuged for 10 min at 25000×*g* after adjusted to pH 5.0 with 5 N acetic acid. An aliquot (2 ml) of the supernatant was filtered by passing and eluting with water in the column packed with 15 ml of Sephadex G-25. Ten ml of eluent after discarding the void volume was collected as the enzyme material.

Epinephrine concentration in plasma was determined fluorometrically in Amberlite IRC-50 eluent according to the procedure of Viktora and Chang.[10,11]

The effect of epinephrine on xanthine oxidase activity *in vitro* was investigated as follows. One ml of Krebs–Ringer bicarbonate buffer was pipetted into the spectrophotometer cell, enzyme solution, epinephrine solution, and water were added, and the volume was adjusted to 2.0 ml. One ml of xanthine solution was then added and the increase of optical density at 290 mμ was recorded for the determination of xanthine oxidase activity.

With other identical mixture, the increase of optical density at 485 mμ was measured for the assay of adrenochrome produced during the reaction period. The net increase of 290 mμ due to the formation of uric acid from xanthine was obtained as a calculated value, which was given as the value deducted the optical density at 290 mμ due to the formation of adrenochrome, corresponding to 2.4 times of 485 mμ value.

Results

Elevation of Plasma Uric Acid on Administration of *l*-Epinephrine

As shown in Fig. 1, a pronounced hyperuricemic state was induced within two hours after intraperitoneal injection of *l*-epinephrine 1.5 mg/kg in young male rats.

In the previous communication by Imaizumi, *et al.*, the plasma uric acid level was determined more than ten hours after administration of epinephrine, so the initial elevation was not observed. Fig. 2 shows the dose response of uric acid level to epinephrine, values being measured one hour after administration. Elevation in uric acid level was higher in older than in younger rats, though this may have been in part due to difference in the absolute amounts given to the two groups.

Elevation of plasma uric acid level was also recognized after intravenous administration of *l*-epinephrine to beagle dog (Fig. 3).

In this case, maximum elevation was reached sooner than in rats given intraperitoneally, a dose response effect being again clearly seen.

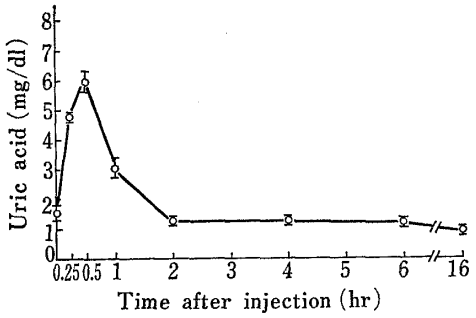

Fig. 1. Plasma Uric Acid Level after Administration of *l*-Epinephrine in Young Male Rats

animals: male rats at 50 days old
l-epinephrine: 1.5 mg/kg, intraperitoneally
Vertical lines represent standard error (n=8).

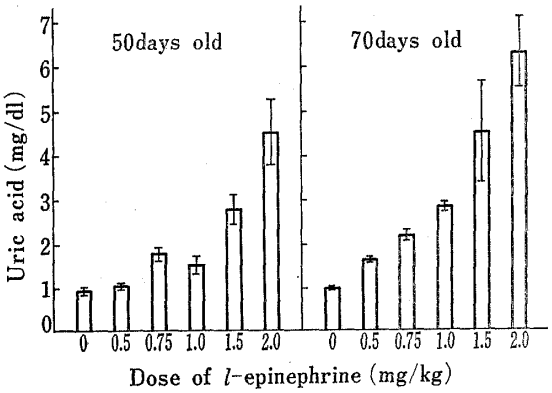

Fig. 2. Plasma Uric Acid Level One Hour after Intraperitoneal Administration of *l*-Epinephrine in Male Rats

Vertical lines represent standard error (n=6).

8) R.M. Archibald, *Clin. Chem.*, 3, 102 (1957).
9) H.M. Kalcker, *J. Biol. Chem.*, **167**, 461 (1947).
10) J.K. Viktora, A. Baukal, and F.W. Wolff, *Anal. Biochem.*, 23, 513 (1968).
11) C.C. Chang, *Int. J. Neuropharmacol.*, 3, 643 (1964).

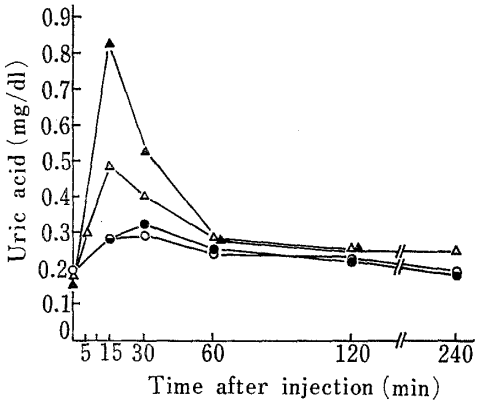

Fig. 3. Effect of *l*-Epinephrine on Plasma Uric Acid in Beagle Dog

l-Epinephrine was administered intravenously.
▲—▲: 0.1 mg/kg △—△: 0.05 mg/kg
●—●: 0.01 mg/kg ○—○: 0.005 mg/kg

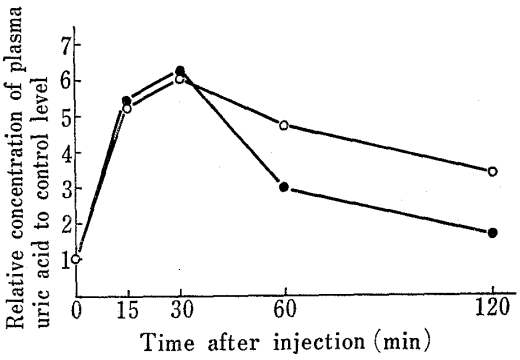

Fig. 4. Elevation of Plasma Uric Acid and Epinephrine after Administration of *l*-Epinephrine in Young Male Rats

l-Epinephrine 1.5 mg/kg was administered intraperitoneally.
●—●: uric acid ○—○: epinephrine
Each plot shows the value calculated from the mean of 5 rats.

A similar induction of hyperuricemia was also observed in mice and guinea pigs administered epinephrine intraperitoneally. An interesting relation noticed between the plasma concentrations of uric acid and epinephrine in rats and dogs was that, while the plasma levels of uric acid and epinephrine in intact dogs are much lower than those in rats, the stimulated levels after injection of 0.1 mg/kg of *l*-epinephrine in dog correspond nearly to the intact levels of epinephrine and uric acid in rats.

This correlation suggested that the plasma level of uric acid is closely correlated to that of epinephrine, so, we next compared the rates of elevation of uric acid and epinephrine level after epinephrine treatment in rats. The absolute concentration of uric acid was always a thousand-fold higher than that of epinephrine, but the rate of elevation after exogenous epinephrine was the same for both substances from the time of administration until the maximum level was reached, the return to normal level, however, was quicker with uric acid than epinephrine (Fig. 4).

When epinephrine was injected repeatedly once an hour, although the plasma epinephrine level increased accordingly, increments of increase in the uric acid level decreased with repeated injection. Thus, as shown in Fig. 5, the increase in uric acid level due to the fourth epinephrine injection was much lower than that after the first injection. Furthermore, the pretreatment of monoamine oxidase and catechol-O-methyl transferase inhibitors for the enhancement of epinephrine action failed to make the more drastic hyperuricemia as seen in Fig. 6.

From this evidence, it appears that exogenous epinephrine has two mechanisms of action; first stimulation of the initial elevation of plasma uric acid, and then repression of this hyperuricemic state. This would correspond to the regulatory mechanism in a long chain metabolic pathway, comprising activation of the enzyme involved in the initial step, then inhibition by the product formed.

Relationship between Epinephrine-induced Hyperuricemia and Adrenergic Receptors

Mimic elevation of plasma uric acid was caused by administration of *l*-norepinephrine, but DOPA, dopamine and metanephrine did not induce hyperuricemia. Isoproterenol gave a much weaker action than that of epinephrine, and this was not dose responsive. These facts suggest an *alpha* response to catecholamine in epinephrine-induced hyperuricemia. Indeed, an α-blocker phenoxybenzamine well inhibited the elevation of uric acid induced by epinephrine (Fig. 7), but a β-blocker dichloroisoproterenol did not (Fig. 8).

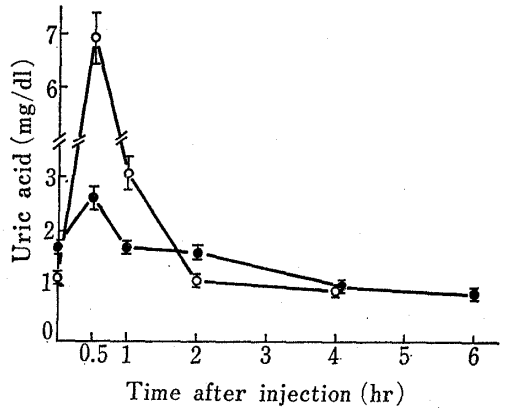

Fig. 5. Plasma Uric Acid Level after Administration of *l*-Epinephrine in Young Male Rats

l-Epinephrine 1.0 mg/kg was administered intraperitoneally.
○—○: after 1st injection
●—●: after 4th injection, with an hour interval
Vertical lines represent standard error ($n=6$).

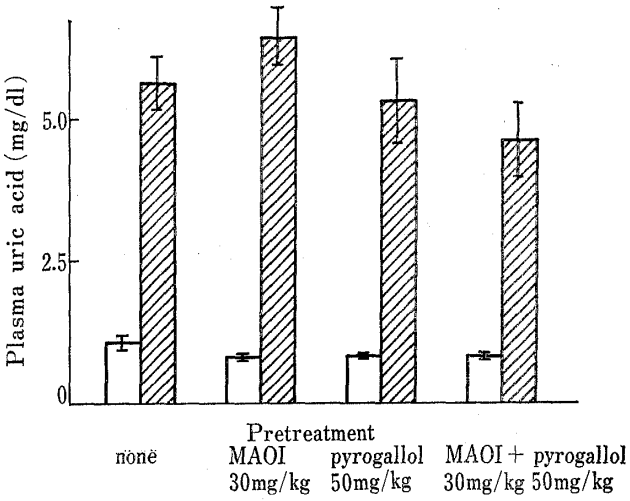

Fig. 6. Effects of Monoamine Oxidase and Catechol-O-methyl Transferase Inhibitors on Epinephrine-induced Hyperuricemia in Young Male Rats

Pretreatment was done intraperitoneally an hour and a half before intraperitoneal administration of *l*-epinephrine.
Plasma uric acid levels are those a half an hour after epinephrine or two hours after pretreatment.
MAOI used was 1-benzyl-2-(3-methylisoxazol-5-yl) carbonylhydrazine.
□: control
▨: *l*-epinephrine administered (1.0 mg/kg)
Vertical lines represent standard error ($n=6$).

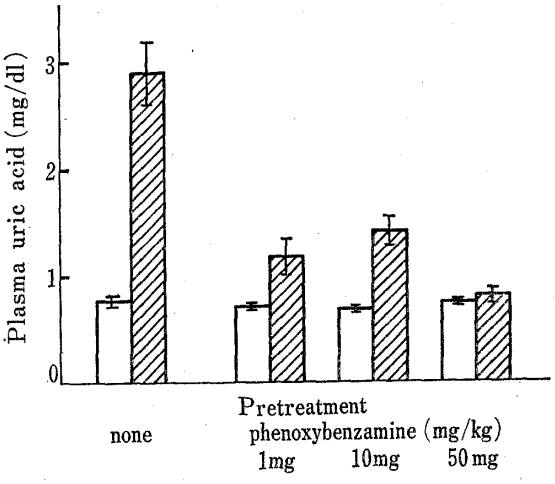

Fig. 7. Effects of Phenoxybenzamine on Epinephrine-induced Hyperuricemia in Young Male Rats

Pretreatment was done intraperitoneally an hour and a half before intraperitoneal administration of *l*-epinephrine.
Plasma uric acid levels are those a half an hour after epinephrine or two hours after phenoxybenzamine.
□: control
▨: *l*-epinephrine administered (1.0 mg/kg)
Vertical lines represent standard error ($n=6$).

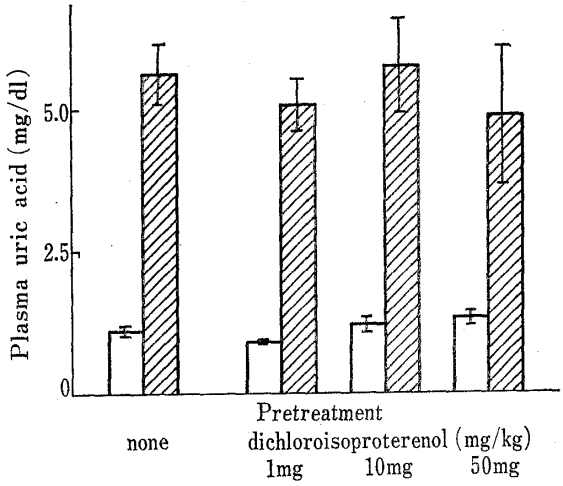

Fig. 8. Effects of Dichloroisoproterenol on Epinephrine-induced Hyperuricemia in Young Male Rats

Pretreatment was done intraperitoneally an hour and a half before intraperitoneal administration of *l*-epinephrine.
Plasma uric acid levels are those a half an hour after epinephrine or two hours after dichloroisoproterenol.
□: control
▨: *l*-epinephrine administered (1.0 mg/kg)
Vertical lines represent standard error ($n=6$).

The effects of pretreatment with various adrenergic blockers were next investigated. Dibenamine, tolazoline and dihydroergotamine inhibited epinephrine-induced hyperuricemia, though their effects were weaker than that of phenoxybenzamine. Dichloroisoproterenol, propranolol and pronethalol did not show such inhibitory effects, and rather they often en-

hanced the epinephrine effect. The most potent antihyperuricemic effect was shown by phenoxybenzamine, which was one hundred times as powerful as dibenamine and tolazoline in this experiment. Moreover, as in no case did the blockade influence the intact level of plasma uric acid, the hyperuricemia induced by epinephrine must be a result of stimulation of the adrenergic α-receptor.

Effect of ATPase Inhibitor on Epinephrine-induced Hyperuricemia

Ouabain is well known as a specific inhibitor of membrane ATPase which is activated by sodium and potassium. Pretreatment with ouabain, like that with adrenergic blockers, caused a marked reduction in both epinephrine-induced hyperuricemia, as shown in Fig. 9, and in the intact level of plasma uric acid. If this ouabain effect is due to the inhibition of membrane ATPase, it may be considered according to the model of α-receptor by Belleau[12] that the epinephrine-induced hyperuricemia is a result of stimulation of membrane ATPase activity.

The enzymic oxidation of epinephrine to adrenochrome has been reported by several workers,[13–15] and the report of Inchiosa[15] makes an important suggestion germane to our consideration. His communication on epinephrine oxidase reports that the oxidative product, produced *via* adrenochrome in this enzyme reaction, is a powerful inhibitor of myosin ATPase. In the present experiment, epinephrine-induced hyperuricemia was remarkably depressed by pretreatment with adrenochrome, as shown in Fig. 10. This agent was excreted in the urine immediately after injection, so the period of pretreatment was defined as shorter than for α-blockade or ouabain treatment.

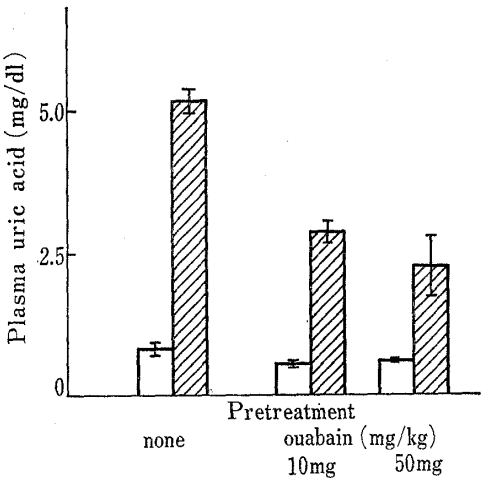

Fig. 9. Effects of Ouabain on Epinephrine-induced Hyperuricemia in Young Male Rats

Pretreatment was done intraperitoneally an hour and a half before intraperitoneal administration of *l*-epinephrine.
Plasma uric acid levels are those a half an hour after epinephrine or two hours after Ouabain.
□: control
▨: *l*-epinephrine administered (1.0 mg/kg)
Vertical lines represent standard error ($n=6$).

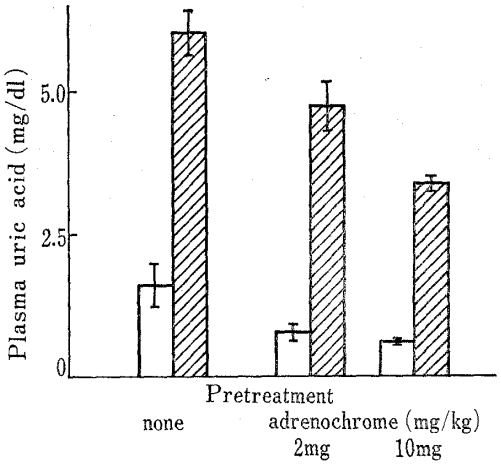

Fig. 10. Effects of Adrenochrome on Epinephrine-induced Hyperuricemia in Young Male Rats

Pretreatment was done intraperitoneally a half an hour before intraperitoneal administration of *l*-epinephrine.
Plasma uric acid levels are those a half an hour after epinephrine or an hour after adrenochrome.
□: control
▨: *l*-epinephrine administered (1.0 mg/kg)
Vertical lines represent standard error ($n=6$).

On the other hand, aminophylline, an inhibitor to phosphodiesterase, did not reduce the uric acid level elicited by epinephrine, so it seems to be likely that the catabolism of ATP induced by epinephrine is mainly proceeded through the ATPase reaction.

12) B. Belleau, *Pharmacol. Rev.*, **18**, 131 (1966).
13) O. Walaas, E. Walaas, F. Henrikson, and R. Lovstad, *Arch. Biochem. Biophys.*, **95**, 151 (1961).
14) Z.L. Hegedus and M.D. Altschule, *Arch. Biochem. Biophys.*, **126**, 388 (1968).
15) M.A. Inchiosa, *Biochem. Pharmacol.*, **16**, 329 (1967).

Effect of Xanthine Oxidase on Epinephrine-induced Hyperuricemia

Imaizumi, *et al.* concluded that activation of xanthine oxidase in the liver is the main cause of epinephrine-induced hyperuricemia. However, no change was observed on xanthine oxidase activity in the liver extract after administration of *l*-epinephrine, while there was marked increase of plasma uric acid (Table I).

Table I. Effect of *l*-Epinephrine on Xanthine Oxidase Activity in Liver Extract and Plasma Uric Acid in Rat

Time after injection min	Xanthine oxidase activity units/mg protein	Plasma uric acid mg/dl
0	1.62±0.15	1.23±0.10
15	1.47±0.11	5.26±0.30[a)]
30	2.01±0.14	7.05±0.35[a)]
60	1.73±0.10	2.76±0.35[a)]
120	1.60±0.10	1.20±0.09

$n=6$, a) $p<0.01$, *l*-epinephrine: 1.5 mg/kg, *i.p.*

On the contrary, supression of xanthine oxidase activity by pretreatment with allopurinol caused a similar decrease in both the hyperuricemic state and the intact level of plasma uric acid (Fig. 11). From this evidence, it seems likely that the modification of xanthine oxidase activity leads to a similar change of uric acid level as brought about by ouabain modification of ATPase activity.

On the other hand, the fact that this enzyme was activated by epinephrine *in vitro* was due to the coupling oxidation of epinephrine to adrenochrome with xanthine.[16] This coupling oxidation proceeded in Krebs–Ringer bicarbonate buffer, as shown in Fig. 12, with the enzyme material from rat liver partially purified by ammonium sulfate fractionation and DEAE-cellulose chromatography.

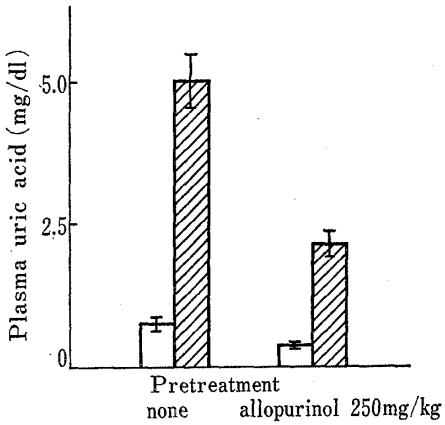

Fig. 11. Effect of Allopurinol on Epinephrine-induced Hyperuricemia in Young Male Rats

Pretreatment was done intraperitoneally 23 hours and a half before intraperitoneal administration of *l*-epinephrine.
Plasma uric acid levels are those a half an hour after epinephrine or 24 hours after allopurinol.
□: control
▨: *l*-epinephrine administered (1.0 mg/kg)
Vertical lines represent standard error ($n=6$).

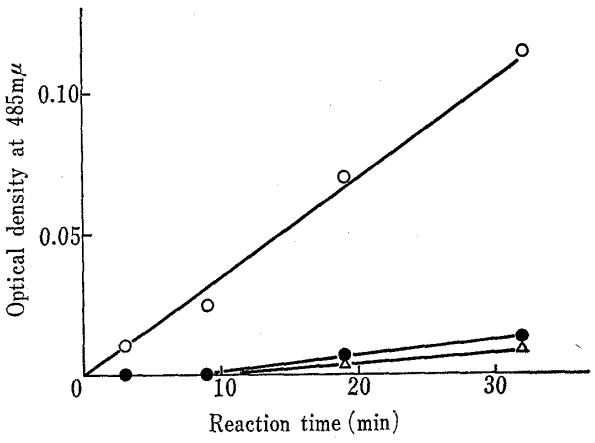

Fig. 12. Coupled Oxidation of Epinephrine with Xanthine Oxidase Reaction

l-epinephrine (A): 5×10^{-4}M
xanthine oxidase (B): 3.0 units
xanthine (C): 0.6×10^{-4}M
○—○: A+B+C ●—●: A+B △—△: A

16) D.M. Valerino and J.J. McCormack, *Fed. Proc.*, **28**, 545 (1969).

The oxidation of epinephrine to adrenochrome was not recognized by the enzyme material, but it was clearly proceeded in the system coupled with substrate of xanthine oxidase. In this coupled system, the enzyme activity was not always activated by epinephrine, but often inhibited. Therefore, it is considered that *in vitro* effect of epinephrine to xanthine oxidase is impossible to support the activation of enzyme *in vivo*.

Discussion

In most considerations on abnormal purine metabolism so far reported, interest has centered on the *de novo* and salvage synthesis of purine nucleotides, and on the reduction of kidney function.[5] In contrast to this, we have presented here a new consideration, that the increased level of plasma uric acid in epinephrine-induced hyperuricemia may result from stimulation of ATP-degradation *via* adrenergic α-receptor. An evidence for the possibility of hyperuricemia induced through the catabolism of ATP was already documented in the case of fructose-induced hyperuricemia.[17]

A possible elucidation for the mechanism of epinephrine-induced hyperuricemia may be given from the concepts on adrenergic receptors by Belleau[12] or Smythies,[18] who clearly classified them on their subsequent biochemical reactions containing the use of ATP in both stimulation of α-receptor and that of β-receptor. They suggested that the stimulation of α-receptor leads to elicit the ATPase reaction, while adenylcyclase reaction is stimulated *via* the action of catecholamine to β-receptor. Our experimental results were mostly well explained by their receptor concepts.

However, another consideration on the mechanism of epinephrine-induced hyperuricemia may be possible, as Demartini[19] documented that the vasoconstriction in kidney by α-acting catecholamines may be one of the reasons to induce the hyperuricemic state. We have tried to examine this possibility by estimating the plasma uric acid levels in the blood, which was collected from various part of vessels under epinephrine administration. The results, which will be presented in our succeeding paper, showed that the increase of uric acid with epinephrine administration was most remarkable in the portal vein and the difference of uric acid concentration between in the blood from artery and in that from abdominal vein is hardly altered by the treatment. Therefore, the renal vasoconstriction by itself can not be considered as a principal cause of epinephrine-induced hyperuricemia.

The concentration of ATP in various tissues was reported to be several ten times of plasma uric acid level, and to be degraded rapidly after ischemia.[20] Our succeeding study, which will be reported elsewhere, also showed that the plasma uric acid in rats rapidly increases during hypoxia caused by nitrogen gas, and this easily returns to normal level after the removal of the gas. Thus, the epinephrine-induced hyperuricemia could be understood as a result of hypoxia caused by the vasoconstriction *via* α-action of epinephrine. This consideration is also supported from the facts that the epinephrine action *via* α-adrenergic receptor comes out in administration of relatively large dose *in vivo*, and the pretreatment with α-blockers obviously inhibits the local vasoconstriction by epinephrine.

A more detailed mechanism of the epinephrine-induced hyperuricemia should be further studied, because of being remained many suspectable problems in the α-action of epinephrine.

Finally, it could be concluded, from the results described above, that uric acid production may be induced more or less by the stimulation of both adrenergic receptors, but a marked hyperuricemic state is elicited only through the α-receptor stimulation, subjecting to the ATPase reaction.

17) K.O. Raivio, M.P. Kekomaki, and P.H. Maenpaa, *Biochem. Pharmacol.*, **18**, 2615 (1968).
18) J.R. Smythies, *Eur. J. Pharm.*, **19**, 18 (1972).
19) F.E. Demartini, *Arth. Rheum.*, **8**, 823 (1965).
20) B. Deuticke and E. Gerlach, *Pflugers. Archiv. ges. Physiol.*, **292**, 239 (1966).