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Abstract

We have designed to apply 2-pyridine aldoxime methiodide(PAM), considered to be a cholinesterase reactivator in vitro, both to the laboratory rabbits poisoned by parathion and to the patients of parathion poisoning, and obtained the following results: 1. With administration of PAM, a prompt and complete dispersion of symptoms of the poisoning can be realized. 2. Cholinesterase activity of red blood cell has instantly and completely recovered, and that of serum transiently. 3. The amount of serum mucoprotein and the activity of active protein-SH-radical of serum varied in direct proportion to the activity of serum cholinesterase. 4. Generally, an intravenous injection of 1g. PAM is sufficient even in the severe case and it may be increased when necessary. 5. The ill effect has not been encountered in the PAM administration. 6. PAM exerts no influence on the cholinesterase activity of normal blood. 7. PAM is expected to play an important role as a prophylactic agent of alkylphosphate poisoning. From these results it seems clear that PAM is a specific and effective antidote against alkylphosphate intoxication.

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CAUSAL THERAPY OF ALKYLPHOSPHATE POISONING

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Of many alkylphosphate compounds synthesized in past twenty years, some have come to be used in practice, as remedies (DFP etc.) and as insecticides (TEPP, parathion, diazinon, etc.) especially after the Second World War[10].

In Japan, parathion has been widely used as an agricultural insecticide mainly on rice plant since 1952, and its use has been encouraged by the Ministry of the Agriculture and Forestry in order to increase rice crop. It is a concensus opinion that parathion as an insecticide has been in a large measure responsible for the bumper crop of rice in 1955 in Japan. In a way useful as it is, parathion also has its short-coming, namely, to human beings, the estimated number of its victims during past five years is said to be over 6,000. Consequently, many studies have so far been made on the poisoning of alkylphosphate compounds, and on the basis of numerous clinical observations[5], the present authors[6] established a standard atropine treatment, by which many patients of parathion poisoning have been saved in Japan. However, it is to be regretted that atropine is not omnipotent.

Recently Prof. I. B. WILSON of Columbia University, introduced 2-pyridine aldoxime methiodide (PAM) as a specific reactivator of acetylcholinesterase inactivated by alkylphosphate. PAM successfully activated the inhibited acetylcholinesterase *in vitro*[12], and hundreds of mice injected with lethal doses of alkylphophate were spared of their lives by PAM injection[8]. But the acting mechanim of PAM *in vivo*, has not yet been persued in full detail and especially so with its clinical effect[15].

The present paper is intended to report experiments in laboratory rabbits and the therapeutic effects of PAM on the patients suffering from acute parathion poisoning.

Method

PAM used is a yellow rhombic crystal with a melting point at 219°C. It is soluble in water 5% at maximum and practically insoluble in alcohol. The structural formula assigned to PAM is shown in Fig. 1.

PAM itself has no power to hydrolyse acetylcholine *in vitro*. The LD_{50} (mg./kg.) of PAM on mice was estimated as 136 ± 6 [8] and 209 (187—233)[14] in the case of intraperitoneal injection, 140 (125—157)[14] subcutaneous injection. In the present study, 5% PAM solution in water was used.

Blood cholinesterase activity was assayed manometrically with TAMAI's modification[11] of AMMON's method[1] at 38°C in a medium containing 0.124M NaCl, 0.025M $NaHCO_3$, 0.0025M KCl, 0.0018M $CaCl_2$ and 0.025M or 0.0025M (the former for human serum cholinesterase and the latter for the others) acetylcholine as substrate. The estimation of cholinesterase activity was based on CO_2 volume produced during 30 minutes after the addition of substrate.

The determination of mucoprotein and active SH-radical of protein were performed polarographically.

Laboratory Experiments

White male rabbits each weighing about 2 kg. were used. Introduction of PAM itself had no influence on the general appearance and blood cholinesterase in rabbits.

A subcutaneous injection of 10 mg./kg. of parathion emulsion (approximate LD_{50}) caused the inhibition of blood cholinesterase and presented the symptom of poisoning such as muscular fasciculation, salivation and noisy respiration.

An intravenous injection of PAM in the rabbits priorly injected with parathion emulsion presented a striking reactivation of blood cholinesterase and the disappearance of symptoms in a short period of time. Twenty four hours after the parathion injection, however, this rabbit died owing to the reinhibition of blood cholinesterase, proving the effect of PAM to be only transient (Fig. 2).

Repeated injections of relatively large dose of PAM presented the maintenance of blood cholinesterase in an activated state, and no symptoms could be observed (Fig. 3), prolonging the life of rabbit even in as high a dosage as 50 mg./kg. parathion injection. In this case, the first PAM injection was the most effective in reactivating cholinesterase but later injections gradually lost their effectiveness (Fig. 4).

In these experiments, PAM was injected in the process of cholinesterase inhibition by parathion and the effect was observable only transiently; but in the cases receiving PAM in the course of natural recovery of cholinesterase activity the cholinesterase reinhibition appearing later was slight (Fig. 5). In both subcutaneous injection (Fig. 6) and oral adminis-

tration (Fig. 7), PAM demonstrated itself just as effective. Oral administration of PAM could likewise protect the cholinesterase inhibiting action of parathion (Figs. 8, 9).

Case Report

Parathion poisoning is divided into four stages, namely, serious, moderate, slight, and latent, by the present authors[6]. This classification is now being used widely in Japan.

Case 1: A serious case, admitted Sept. 2, 1956.

A 17-year old high school boy, sprayed a rice field with a parathion solution. On the next day he became ill in the evening. After receiving 20-ampule injection of atropine, 0.5 mg. each, the patient was brought to our clinic 3 hours after the onset of symptom. He then was in the convalescent stage.

An intravenous injection of 0.1 g. PAM produced neither changes of symptoms nor toxic effect, but the transient and slight recovery of red blood cell cholinesterase (Fig. 10). This boy improved 6 hours after the onset of poisoning.

Case 2: A serious case, admitted Sept. 2, 1956.

A 24-year old farmer, sprayed a rice field with a parathion solution. In the afternoon of the day after this work, he fell ill complaining of nausea, vomiting, abdominal cramps, diarrhea, profuse sweating, salivation, involuntary micturition, muscular fasciculation and coma.

He was given ten intravenous injections of 0.1 g. PAM each during the periods of 3 and a half hours. By the time of the last PAM injection, all the symptoms had disappeared and no toxic effect of PAM itself could be discernible. In this case, the recovery of blood cholinesterase activity were impressive, in that red cell cholinesterase being reactivated by degrees, reached over normal. On the contrary, serum cholinesterase activity moved slightly, though it only required half the time for the recovery to the normal level compared with the time required by the untreated (Fig. 11).

Case 3: A serious case, admitted Sept. 3, 1956.

This 24 year old farmer had been engaged in spraying a rice field with a parathion solution. On the 3rd day he fell ill and was admitted to our clinic. He then complained of dizziness, nausea, vomiting, headache, salivation, profuse sweating and slight muscular fasciculation.

On admission 0.5 g. PAM was injected intravenously without any

toxic sign; and therefore, the further injection of 0.4 g. PAM was made 10 minutes later. Immediately after the second injection, all his complaints disappeared. Complete recovery of red cell cholinesterase took place 40 minutes after the last PAM injection, though serum cholinesterase activity made a very slight recovery, and returning to normal in 15 days (Fig. 12).

Case 4: A serious case, admitted Sept. 6, 1956.

A farmer, age 28. This case is supposed to be the first of parathion poisoning ideally treated with PAM, and is, therefore, presented in detail.

History: Engaged in spraying a rice field with 0.1% parathion solution. At 1:30 p. m. on the 3rd day of the work, he had dizziness, profuse sweating, salivation and tightness of the chest, but continued to work until 3:00 p. m., when nausea and vomiting began. Shortly thereafter, he went home and thoroughly washed himself. At 4:00 p. m. he was attacked by severe dizziness, slurred speech, blurring of vision, and coma. After the injection of 3 ampules of atropine, he was brought to our clinic at 6:30 p. m.

Physical Examination: Well developed male, seriously ill and unresponsive. His respiration was slow and seemed difficult. The pulse rate was 130 per minute, and blood pressure 170/110 mm. Hg. The body temperature 37.5°C. Muscular fasciculation was very marked and generalized. He was no longer sweating but had excessive salivation. The pupils were pinpoint in size, reacting scarcely to light. The cardiac dullness was slightly enlarged with the heart sounds powerful and good. The lung and abdomen were normal. Tendon reflexes of the extremities were absent.

Laboratory Findings: Cholinesterase activity of red blood cell was 28.9 ml. CO_2, approximately 13% of normal, and that of serum 4.9 ml. CO_2, only 0.6% of normal. Serum mucoprotein was 38.8% and SH-radical activity of serum protein 47.5% of normal. Leucocyte count was 19,900 per cubic millimeter, with 87% polymorphonuclear cells, 10% lymphocytes and 3% monocytes. Eosinophiles were absent. Hemoglobin was 12.6 g./dl., with 5,170,000 erythrocytes per cubic millimeter. Urine definitely contained sugar, slight urobilinogen and 133.8 mcg/dl. of p-nitrophenol. Blood sugar 149 mg./dl. Cephalin cholesterol flocculation, Takata's test, icterus index and other tests on serum were normal. Electrocardiogram showed right bundle-branch block, probably related with poisoning.

Course and Treatment: At 6:37 p. m. 7 minutes after admission he was given 1 g. of PAM intravenously. The muscular fasciculations diminished instantly except in certain parts of the breast and legs and he

was awake and responsive.

At 6: 41 p. m. blood pressure was 158/80 mm. Hg. Respiration quieted down. At 6 : 58 p. m. 21 minutes after PAM injection, the muscular fasciculation disappeared completely and no symptoms could then be found.

The hourly changes of the blood cholinesterase values are given in Fig. 13. The activity of erythrocyte cholinesterase returned to normal within 10 minutes of PAM injection, serum cholinesterase reactivated transiently, but returned to normal earlier than that of untreated case.

The changes of mucoprotein and protein-SH-radical activity of serum are given in Fig. 14. Both show the similar changes as those of serum cholinesterase. In other cases, serum mucoprotein and protein-SH-radical activity of serum are similar to this case.

Case 5: A serious case, visited Sept. 10, 1956.

A 37 year-old farmer, who had consulted a doctor about a week before, visited our clinic on the 8th day of acute parathion poisoning. He was then complaining only of slight headache with no physical findings. Blood cholinesterase activity was also on the way to recovery.

Intravenous injection of 1 g. of PAM was immediately given, but he felt discomfort less than one minute and showed no other toxic symptoms. Blood cholinesterase value showed little change (Fig. 15).

Discussion

1. The administration of PAM dispersed promptly the symptom of poisoning:

The effect was more remarkable in the clinical experiments than in the animal experiments.

In the case 1, the clinical improvement was hardly noticeable due probably to a small dosage of PAM. In the case 2, the effect was some what obscure and as for the cases 3 and 4, to whom sufficient dosage of PAM had been administered in a single dose, dramatical effects were observed at the instance of the injection; recovery of a clear consciousness, disappearance of mascular fasciculations, an improvement of respiratory function and so on. In these cases, the dosage of atropine was not large enough to affect the symptoms, except the case 1 who was cured by atrophine rather than by PAM.

The treatment with atropine, which has so far been considered to be an effective medicament, can disperse only a portion of poisoning symptoms, and can not affect symptoms such as mascular fasciculation. Be-

sides, it has its short-coming in that as it requires a large dosage to make it effective, there naturally arise unpleasant side-effects.

With the administration of PAM, however, all the symptoms could be made to disappear and there arose no unpleasant side effects. From this fact it may safely be said that PAM is the specific and the causal therapeutics in the treatment of alkylphosphate poisoning.

2. Cholinesterase activity of red blood cell was instantly and completely recovered, and that of serum gradually:

In only ten minutes after PAM injection, cholinesterase activity of red blood cell recovered to normal as observed in the case 4. Such a phenomenon could not be expected so far. The activity then decreased to some extent but remained within the normal limit in human. This finding is quite surprising since without PAM administration the recovery of cholinesterase activity generally takes quite a long period, a month or more in serious cases and since it is not in any way affected by atropine treatment. The recovery rate of serum cholinesterase activity, however, was slower and transient, but even in such a case, it regained the normal value in about a half of the length of time as compared with the untreated.

There are two types of cholinesterase, true or acetylcholinesterase and non-specific or pseudocholinesterase. In human, the former is contained in the nervous tissue and red blood cells, and is regarded to act on the neurohumoral transmission. The latter is contained in serum, and is considered to have essentially no funciton on the neurohumoral transmission but related closely to the liver function. In the rabbit, serum cholinesterase belongs to acetylcholinesterase. Thus this fact may make it comprehensible why the values of both serum and red cells in the rabbit cholinesterase behave alike while they do not in human cholinesterase, and why all the symptoms do disappear without restoration of serum cholinesterase in patients..

From these results, it is obvious that the main effect produced by the administration of PAM is the restoring of the activity of acetylcholinesterase and the effect on pseudocholinesterase is but complimentary. As the cholinesterase of the nervous tissue is of true type, PAM is remarkably effective in the treatment of alkylphosphate poisoning.

KEWITZ and WILSON rightly assumed that PAM probably reactivate acetylcholinesterase *in vivo*, making their assumption on the basis of their *in vitro* experiments in which it has been found that PAM is a much poorer reactivator of human serum cholinesterase[8].

At the present the definite diagnosis of alkylphosphate poisoning is

being made mainly on the lowering of serum cholinesterase (pseudocholinesterase) value besides physical examinations. It has been generally accepted that the degree of the damage *in vivo* caused by alkylphosphate poisoning may be estimated more exactly by measuring the acetylcholinesterase. We would venture to recommend rather the measuring of serum cholinesterase activity in making qualitative and quantitative analysis of alkylphosphate poisoning, because of the complexity in the determination of the activity of red cell cholinesterase to be used in actual practice and because of a relatively long time required in becoming proficient in the manipulation. Mydriasis, the most standard symptom of the diagnosis, has almost lost its significance in our clinic, as atropine has come to be widely used in the treatment and nearly all the patients are sent to our clinic after the premedication with atropine. In future when PAM treatment attains a universal recognition, it might be possible to diagnose the poisoning after PAM administration basing not on the cholinesterase activity of red blood cell but on that of serum.

3. The behavior of serum mucoprotein varies in direct proportion to the activity of serum cholinesterase and the behavior of active protein SH radical of serum nearly coincides with that of serum mucoprotein:

Changes other than those of cholinesterase activities in parathion poisoning have been recently observed by the present authors with mucoprotein and active protein-SH-radical in serum; and the findings of which are arousing a keen interest in Japan[4]. With PAM administration, the changes of serum mucoprotein are similar to those of serum cholinesterase, and those of active protein-SH-radical are also similar but less remakable.

4. An intravenous injection of 1 g. PAM is generally sufficient but the dosage may be increased if necessary:

Considering the acting mechanisms of PAM and parathion, one molecule of parathion may be thought to combine with one molecule of PAM. As the molecular weight of both substances are nearly equal, it will be sufficient to give PAM in the same proportion of dosage to the amount of parathion, but from our experiments, it seems more advisable to give larger dosage.

As in the cases 2, 3 and 4, an intravenous injection of 1 g. PAM was sufficient to disperse the clinical symptom and restore the cholinesterase activity of red blood cell. After the administration of PAM, a transient decrease of the activity of cholinesterase of red blood cell is apt to occur, and so in serious cases there may be a possibility of relaps of the symptom together with the reinhibition of cholinesterase. But from

our experiences, it is found that if the patient survived for as long as 12—24 hours after the onset of symptoms, he generally recovered spontaneously.

From these facts, 1 g. dosage may be sufficient and if needed one or two more grams of PAM may be given.

Now we are considering of giving larger doses of PAM in order to treat parathion poisoning more effectively as we believe that this seems to be especially necessary with the case where larger amount of parathion is taken as in suicide cases.

Because of the time element involved in the treatment of parathion poisoning, PAM was administered intravenously, but subcutaneous route may be employed as well. Oral administration may be effective but not practical, for parathion poisoning, even in its mild form, is usually accompanied by nausea and vomiting.

5. No ill effect has been encountered in the PAM administration :

The ill effect of any new drug should be seriously taken into consideration. In laboratory mice experiment, the intravenous LD_{50} of PAM was found to be 140 mg./kg. (125—156), and this dosage turned out to be sufficient in the actual application.

Some patients as in the cases of 1 and 2, receiving a smaller dosage of PAM, had vomiting, but it seemed to be the symptom caused by parathion poisoning rather than the drug itself as no such ill effect was found in the patients like cases 3 and 4, given larger doses of PAM. The patient like case 5, receiving rather rapid PAM intravenous injections, complained of a slight discomfort lasting only one minute.

Abnormalities of both blood picture and liver function were not observed. No pains or local changes of vein and skin at the site of the injection appeared. As far as PAM is concerned, these facts seem to assure that there is no danger of ill effect in the conventional administration now in use and even in case of larger dosage.

6. PAM had no influence on the cholinesterase activity of normal blood:

After the administration of PAM, the blood cholinesterase activity in normal rabbits remained within its normal range and this was also the case with that of man, case 5. WIRTH[13] and JENSEN[7] have reported that most of phosphoric acid derived from parathion was excreted within 1—3 days and later only in a small amount. Judging from these facts, it might be assumed that PAM has the activity only against the phosphorylated cholinesterase (Fig. 16).

7. PAM is expected to be used as a preventive agent to alkylphos-

phate poisoning:

As already mentioned, PAM exhibits no effect when intravenously administered in small doses for a prophylactic purpose. This fact is construed to be due to the possibility of PAM being promptly excreted or inactivated. On the other hand, PAM may be assumed to be a preventive agent of the lowering of cholinesterase activity in rabbit by oral administration in a large dosage. In man it is expected to be more effective than in the rabbit as we have seen in its therapeutic uses.

Hitherto, alkylphosphate poisoning has mainly been treated by atropine, but now it should be replaced by PAM. PAM as a causal therapeutic agent to not only the alkylphosphate but also the nerve gas poisonings, we might expect a rapid progress of the study on enzyme mechanisms.

Summary

We have designed to apply 2-pyridine aldoxime methiodide (PAM), considered to be a cholinesterase reactivator *in vitro*, both to the laboratory rabbits poisoned by parathion and to the patients of parathion poisoning, and obtained the following results:

1. With administration of PAM, a prompt and complete dispersion of symptoms of the poisoning can be realized.
2. Cholinesterase activity of red blood cell has instantly and completely recovered, and that of serum transiently.
3. The amount of serum mucoprotein and the activity of active protein-SH-radical of serum varied in direct proportion to the activity of serum cholinesterase.
4. Generally, an intravenous injection of 1 g. PAM is sufficient even in the severe case and it may be increased when necessary.
5. The ill effect has not been encountered in the PAM administration.
6. PAM exerts no influence on the cholinesterase activity of normal blood.
7. PAM is expected to play an important role as a prophylactic agent of alkylphosphate poisoning.

From these results it seems clear that PAM is a specific and effective antidote against alkylphosphate intoxication.

References

[1] AMMON, R.: Pfluegers Arch. Physiol., 233, 486, 1935. — [2] CONLEY, B. E. et al.: J. A. M. A., 144, 104, 1950. — [3] HIRAKI, K. NAMBA, T. et al.: Japanese Med. J., No. 1613, 15, 1955. — [4] HIRAKI, K. NAMBA, T. et al.: Tokyo Med. J., 72, 137, 1955. — [5] HIRAKI K.: Nisseki-igaku, 8, 8, 1955. — [6] HIRAKI, K. NAMBA T. et al.: Japanese Med. J., No.

1636, 7, 1955. — [7] JENSEN, H. DURHAM, W.F. et al.: Arch. Ind. Hyg. Occup. Med., 326, 1952. — [8] KEWITZ, H. and WILSON, I. B.: Arch. Biochem. Biophys., 60, 261, 1956. — [9] NAMBA, T. et al.: Okayama Igakkai Zasshi, 68, 1547, 1956. — [10] SCHRADER, G.: Angew. Chem., 62, 471, 1950. — [11] TAMAI, A.: J. Japan. Biochem. Soci., 22, 32, 1950. — [12] WILSON, I. B. and GINSBURG, S.: Biochem. et Biophys. Acta, 18, 168, 1955. — [13] WIRTH, W.: Deut. Med. Wshr., 79, 1205, 1954. — [14] YAMAMOTO, S. SATO, H. et al.: Personal communication. — [15] Chem. Eng. News, p. 1305 and 1446, 1956.

Explanations of Figures

Fig. 1. Structural formula and crystal of 2-pyridine aldoxime methiodide (PAM).

Fig. 2. Rabbit No. 1. Blood cholinesterase acitivity in comparison with that of untreated case (with PAM). Transitory recovery of cholinesterase activity by PAM injection, i.v.

Fig. 3. Rabbit, No. 2. Blood cholinesterase activity compared with that of the untreated. Continuous reactivation of cholinesterase by repeated PAM injections, i.v.

Fig. 4. Rabbit, No. 3. Blood cholinesterse activity. Prolonged life and recovery of cholinesterase by repeated intravenous injections of large doses of PAM even in the case injected with 50 mg/kg parathion, s.c.

Fig. 5. Rabbit, No. 4. Blood cholinesterase in comparison with that of untreated case. Little reinhibition of cholinesterase activity by an intravenous injection of PAM at the time of the cholinesterase activity while recovering.

Fig. 6. Rabbit, No. 5. The comparison between blood cholinesterase activity and that of the untreated. Transitory reactivation of cholinesterase by a single subcutaneous injection of PAM.

Fig. 7. Rabbit, No. 6. Blood cholinesterase activity compared with that of untreated case. Slower reactivation and reinhibition of cholinesterase by an oral PAM administration.

Fig. 8. Rabbit, No. 7. Blood cholinesterase in comparison with that of untreated case. Slight cholinesterase inhibition by an oral application of PAM 30 minutes before parathion injection.

Eig. 9. Rabbit, No. 8. Blood cholinesterase in comparison with that of untreated case. Slight and slow inhibition of cholinesterase by an oral PAM application simultaneous with parathion injection.

Fig. 10. Case 1. Serious case. Blood cholinesterase compared with untreated case. Transitory slight recovery of red blood cell cholinesterase by an intravenous injection of 0.1 g. of PAM.

Fig. 11. Case 2. Serious case. Blood cholinesterase in comparison with untreated case. Recovery of red blood cell cholinesterase to normal step by step by repeated PAM injections, i.v. a total of 0.1 g.

Fig. 12. Case 3. Serious case. Blood cholinesterase activity in comparison with that of untreated case. Instantaneous recovery of red cell cholinesterase to normal, and slight recovery of serum cholinesterase by PAM injections, i.v., total of 0.9 g.

Fig. 13. Case 4. Serious case. Blood cholinesterase activity in comparison with that of untreated case. Instant and complete recovery of red cell cholinesterase and transient recovery of serum cholinsterase by a single 1.0 g. PAM injection, i.v.

Fig. 14. Case 4. Serious case. Serum mucoprotein and serum protein SH radical activity. Neary similar changes to serum cholinesterase activity.

Fig. 15. Case 5. Serious case. On 8th day of the onset of poisoning. Blood cholinesterase by an intravenous injection of 1.0 g. of PAM, i.v.

Fig. 16. A schematic diagram of the inhibition of cholinesterase by parathion and the reactivation of cholinesterase by PAM.

2-pyridine aldoxime methiodide
(PAM)

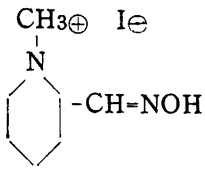

Fig. 1

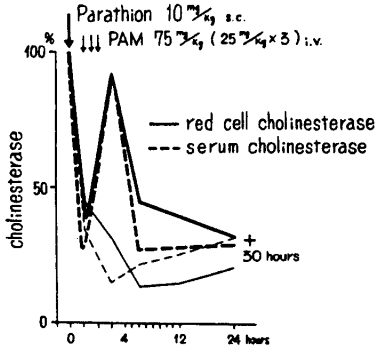

Fig. 2

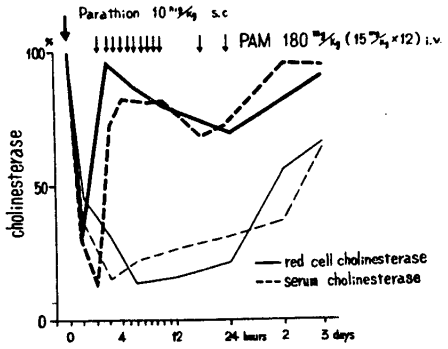

Fig. 3

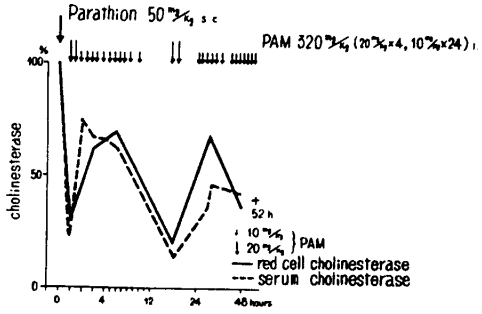

Fig. 4

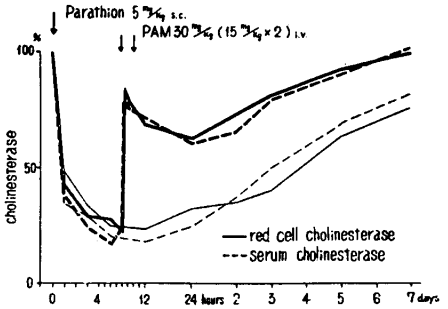

Fig. 5

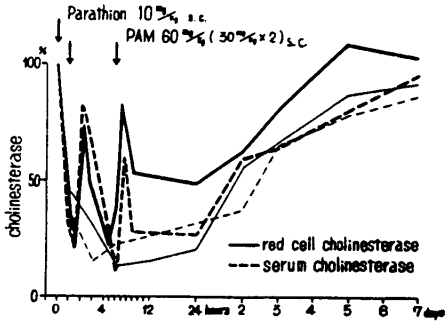

Fig. 6

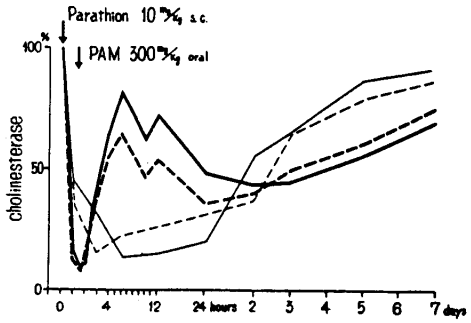

Fig. 7

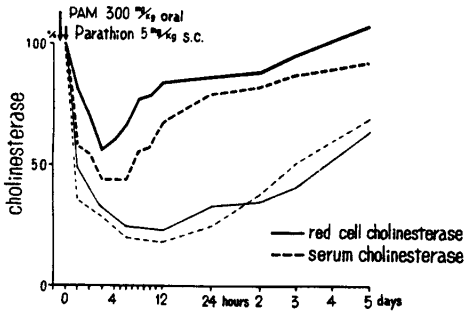

Fig. 8

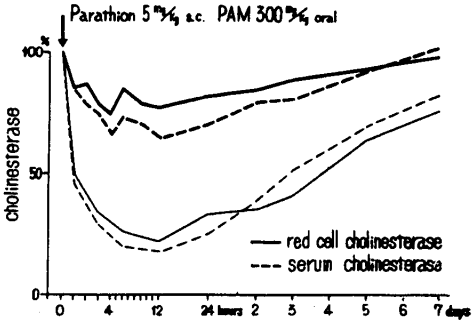

Fig. 9

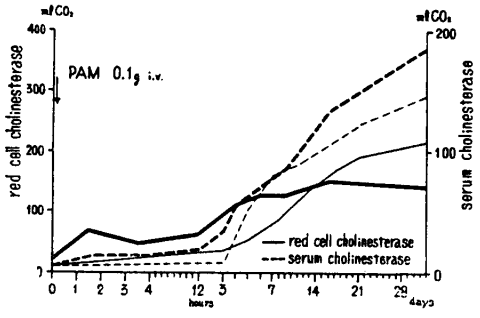

Fig. 10

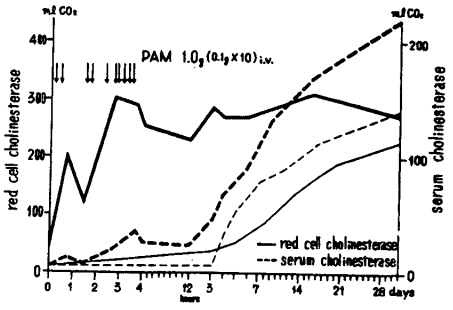

Fig. 11

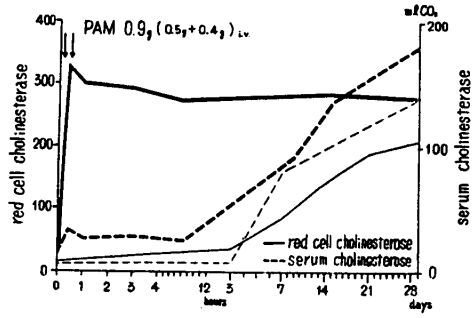

Fig. 12

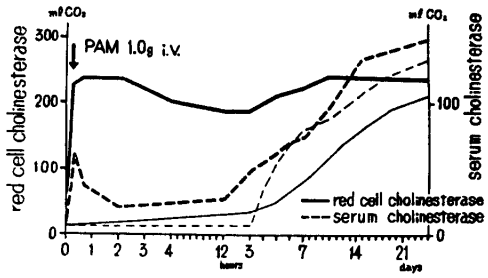

Fig. 13

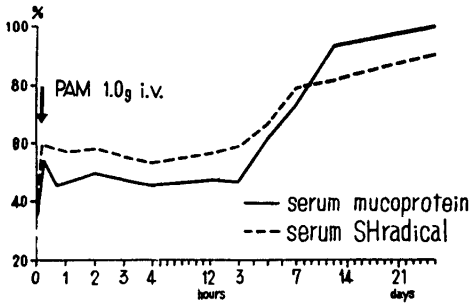

Fig. 14

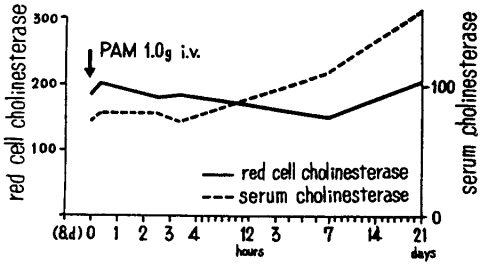

Fig. 15

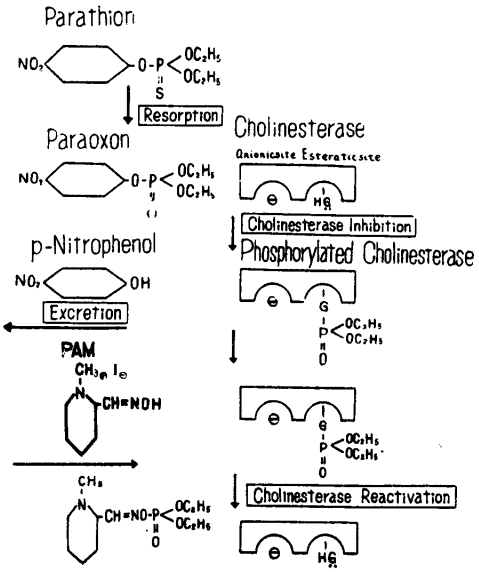

Fig. 16