# Experimental Studies on the Nitroglycol Poisoning

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(Received October 6, 1973)

Nitroglycol has been used in the explosive industry as an anti-freezeer of dynamite before the war. Furthermore, it was already known that the elevation of the mixing rate of nitroglycol is of great advantage in the points capable of aboliting the very dengerously kneading operation of dynamite and of mounting dynamite in safety. However, nitroglycol was impossible to reply to the demand, because it amounted to a high price in the time of the dependence of synthesis of ethylene glycol to alcohol.

In recent years, the striking development of petroleum industry made it possible to purchase ethylene glycol in large quantities and at an inexpensive price and as the result, the utilization of nitroglycol was accelerately promoted. Consequently, the mixing rate of nitroglycol increased steadily and rose up to 50 percent in 1955 and furthermore, to 60 percent in 1958. On the other hand, among the workers engaged in the explosive plant, the patients who complain of different subjective symptoms appeared in succession. The followings are involved as the main symptoms characteristic for the nitroglycol poisoning, that is, headache, vomiting, sholder-discomfort, gastralgia, throat-contracting feeling, chest-compressing feeling, intoxication in the acute poisoning, and the anginal syndrome in the chronic poisoning, which occurs suddenly on the next or second day after holidays. Therefore, the managers in the plant devised a variety of counterplans in consideration of the importance of the nitroglycol poisoning and on this problem, many investigators[1)2)3)4)5)6)] have carried out the wide-spread studies.

In especial, the intoxication, in the course of working, take place through the absorption of nitroglycol vapour and the attachment of nitroglycol to the skin. In most cases of the intoxicated patients, the blood-pressure at the maximum lowers, while in a few cases, the blood pressure at the minimum rises and the pluse-pressure lowers. Such a symptom is cured for a little while in the light state and restores to normal after a night eren in the serious state. On the other hand, the anginal syndrome often occurs when the worker who has been exposed to nitroglycol for a long time is in apart from his work for one or two days. Moreover, it resembles the abstinent phenomena and as a rule, appears on rising from a bed in the morning. At that time, the patients complain of the subjective symptoms, such as stiffness in shoulder and chest-compressing feeling, com-

plexionless, dyspnoea, and unconsciousness; and some of them are said to cause of death. Therefore, the nitroglycol poisoning first was called the Monday disease or the Monday attack. In general, a genuine case of angina pectoris shows the characteristic findings in electrocardiograph. On the other hand, an intoxicated case is named as the anginal syndrome, because one has not such the findings and is generally cured while 3 to 30 minutes.

In foreign countries, the dead cases by the nitroglycol poisoning seem to have appeared fairly before and Symanski[7] has reported that there were the fatal cases of many cases (1927 to 1936) in the United States of America, of 6 to 8 cases (until 1952) in Scotland, and of one case in Western Germany. In our country, since 1959 the patients suspected of the nitroglycol poisoning have appeared and from 1959 to 1960, 4 workers[8] died.

Thereafter, the managers have payed an attention on the healthy managent of workers and carried out the lowering of mixing rate of nitroglycol and the improvement of various conditions, such as exhaust ventilator, aspirator, a suit for protection, and controls of material temperature. As the result, the reports for fatal cases have not been seen since then. Whereas, most of the workers have nowadays complained of different subjective symptoms.

Therefore, the author has developed further studies on the nitroglycol poisoning using the rabbits. This paper deals with the change of free nitroglycol, ethylene glycol, and nitrate content in the blood in 1st chapter; and the formation of methemoglobin and its metabolism in 2nd chapter; and the variation of serum transaminases and catalase activities in the blood in 3rd chapter; and the change of reduced glutathione, lactic acid, and blood sugar content in the blood in 4th chapter.

## I. The Change of Free Nitroglycol, Ethylene Glycol, and Nitrate Content in the Blood

### INTRODUCTION

Most of foreign compounds that are introduced into human and animal bodies there changed chemically. Moreover, throughout the following metabolic pathways, the temporary or only hypothetically intermediate metabolites are produced from them and these metabolites generally give rise to certain poisoning to the bodies. Consequently, if we want to know the mechanism of poisoning for nitroglycol, the unchanged material and its metabolites must be studied together. Nitroglycol is assumed easily to be hydrolyzed in the bodies, because it is ester although it is a pure foreign compound having no relation to physiological ingredients. In addition, in hydrolysis the metabolites of ethylene glycol and nitrate are pressumed to be produced.

Therefore, as a first step of the successive investigation for the nitroglycol poisoning, the author pursued free nitroglycol and the metabolites, such as ethylene glycol and nitrate in the blood using the rabbits.

## MATERIALS AND METHODS

Animals: Healthy, male rabbits weighing from 2.0 to 3.5kg were used and fed on solid rabbit feed.

Materials: Ten % nitroglycol olive solution (Nipponkayaku Co.).

Method of administration of nitroglycol: The rabbits were subcutaneously injected with 1.5 ml of 10 % nitroglycol olive solution (0.15 g as nitroglycol) per kg of the body weight once in a day.

Blood sampling: The blood was punctured from the heart with an injection syringe containing heparin.

Estimation of free nitroglycol in the blood[9]: One and a half ml of pure water and 5 ml of ether were added to 0.5 ml of the blood and shaked rigorously for several minutes, followed by centrifuging for 10 minutes at 7000 r.p.m. (0°C). The ethereal layer was separated out and to this were added 7 ml of ethanol, 1 ml of pure water, and 1 ml of 2 % potassium hydroxide ethanol solution in that order, respectively. Subsequently, the mixture was incubated for 60 minutes at 40°C and allowed to stand at room temperature. After cooling, each 1 ml of 6 N hydrochloric acid and 1 % sulfanilamide solution were added and 3 minutes later, 1 ml of 0.5 % ammonium sulfamate solution was added. After 2 minutes, 1 ml of 1 % N-(1-naphthyl)-ethylene-diamine solution was added and allowed to stand for 10 minutes at room temperature. The optical density was measured against the reagent blank at the wavelength of 530 mμ by spectrophotometer. The content of free nitroglycol was calculated on the basis of the calibration curve and expressed as μg/ml of the blood.

Estimation of nitrate in the blood[9]: To the residual blood from which nitroglycol was extracted, 3 ml of pure water and 2 ml of 8 % trichloroacetic acid were added. The mixture was shaked rigorously, followed by centrifuging for 10 minutes at 7000 r.p.m. (0°C). Five ml aliquot of the supernatant was transferred into a 50 ml-conical flask. The flask was kept for 5 minutes in a ice water bath (0°C) and 5 ml of concentrated sulfuric acid was added slowly. Subsequently, 2 ml of brucine chloroform solution was added to this and the mixture was allowed to stand for several minutes in the dark room. The cholroform was completely evaporated by heating for 20 minutes in an incubator at 90°C. The flask was taken out and allowed to stand at room temperature to cool. The optical density was measured against the reagent blank at the wavelength of 410 mμ. The content of nitrate was calculated from the calibration curve and expressed as μg/ml

of the blood.

Estimation of ethylene glycol in the blood: To 0.5 ml of the blood were added 3.5 ml of pure water, 0.5 ml of 10 % sodium tungstate solution, and 0.5 ml of 2/3 N sulfuric acid in that order, respectively. The solution was rigorously shaked and subjected to centrifugation for 10 minutes at 7000 r.p.m. (0°C). The estimation of ethylene glycol mentioned below was carried out according to the method of Bricker et al.[10] One ml aliquot of the supernatant was transferred into a 10 ml-volumetric flask. To this were added 4 ml of pure water and 1 ml of periodic acid solution (150 mg/ml) and the mixture was swirled occasionally. After 10 minutes, 1 ml of lead acetate solution (100 mg/ml) was added as soon as possible in order to prevent the reaction from further proceeding. The solution was filled up to 10 ml with pure water, followed by centrifuging for 5 minutes at 1600 r.p.m. One ml aliquot of the supernatant was pipetted into a test tube and to this were added 0.5 ml of chromotropic acid solution (100 mg/ml) and 6 ml of concentrated sulfuric acid. The mixture was heated in a boiling water for 30 minutes and then, immersed into running tap water. After cooling, the contents of test tube was transferred into a 25 ml-volumetric flask and the volume was made to 25 ml with pure water. The optical density of the solution was measured against the reagent blank at the wavelength of 570 mμ. The content of ethylene glycol in the blood was calculated on the basis of the calibration curve and represent in terms of μg of ethylene glycol per ml of the blood.

## RESULTS

The change of free nitroglycol content in the blood:

The rabbits were subcutaneously received 1.5 ml of 10 % nitroglycol olive solution (0.15 g as netroglycol) per kg of the body weight. The change of free nitroglycol content in the blood was observed with a lapse of time after the injection (Fig. 1).

The depressing and ascending curve of free nitroglycol content in the blood showed the about same tendency in another case except one case of No. 4. Namely, the content of free nitroglycol in the blood increased remarkably as soon as nitroglycol was injected and reached the maximum at 1 hour later. Thereafter, it decreased immediately, fell to the trace amount at 6 hours later, and at 24 hours later, could not be detected in the blood at all. The maximum concentrations of free nitroglycol in the bloods of the rabbits were as follows: 26.60 μg/ml in No. 1, 6.98 μg/ml in No. 2, 31.14 μg/ml in No. 3, 12.28 μg/ml in No. 4, 24.20 μg/ml in No. 5, respectively.

The change of nitrate content in the blood:

The content of nitrate in the blood was determined using the residual blood from which nitroglycol was extracted and it was plotted with time after the injection (Fig. 2).

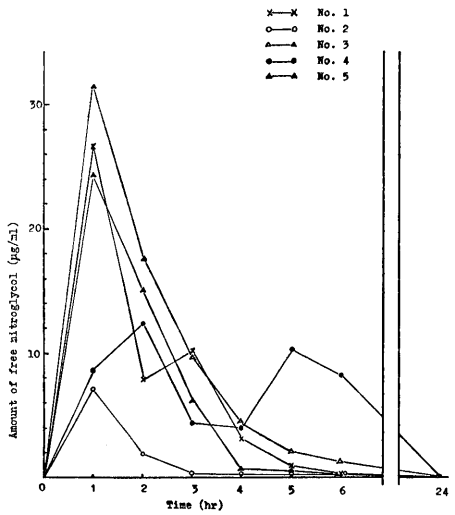



Fig. 1. The change of nitroglycol content in the blood after the injection of nitroglycol.

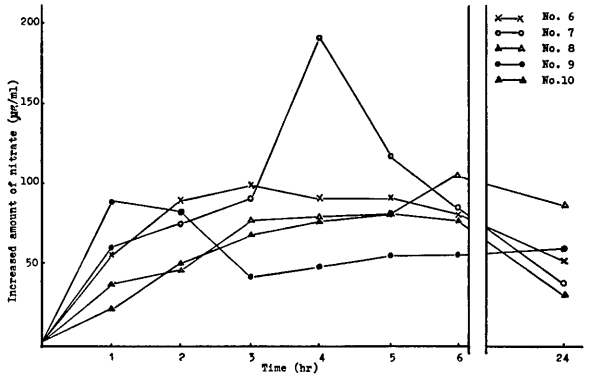



Fig. 2. The change of nitrate conteut in the blood after the injection of nitroglycol.

Nitrate is one of the common ingredients in the blood and always detected in some quantities already in the blood before the injection of nitroglycol. Therefore, the content of nitrate in the blood in Fig. 2 shows the increasing amount, that is, the value deducting the level before the injection from that after the injection. The concentrations of nitrate in the bloods of the rabbits were as follows: 20.52 μg/ml in No. 6, 21.25 μg/ml in No. 7, 22.60 μg/ml in No. 8, 24.27 μg/ml in No. 9, and 30.94 μg/ml in No. 10.

The depressing and ascending curve of nitrate in the blood indicated the somewhat similar tendency in other cases except two cases of Nos. 9 and 7. Namely, the content of nitrate in the blood gradually increased during 3 hours after the

injection of nitroglycol and was approximately constant from 3 to 5 hours later. Thereafter, it began to decrease very slowly, but at 24 hours later, did not fall to the pre-injection level. The increasing rate of nitrate at 24 hours later was as follows; 2.27 in No. 6, 2.82 in No. 7, 4.35 in No. 8, 3.35 in No. 9, and 2.07 in No. 10.

The change of ethylene glycol in the blood:

The rabbits were treated as well as nitroglycol and nitrate, and the change of ethylene glycol content in the blood was observed with lapse of time after the injection (Fig. 3).

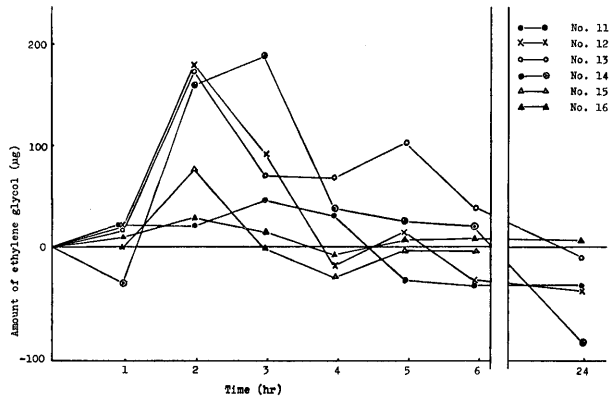



Fig. 3. The change of ethylene glycol content in the blood after the injection of nitroglycol.

The determination of ethylene glycol on the basis of chromotropic acid method is not necessarily specific for ethylene glycol. As the result, other polyhydric alcohol give rise to the same reaction as ethylene glycol. The concentrations of ethylene glycol in the blood of each rabbit before the injection were as follows; 42.86 μg/ml in No. 11, 38.40 μg/ml in No. 12, 42.30 μg/ml in No. 13, 96.00 μg/ml in No. 14, 48.00 μg/ml in No. 15, and 21.00 μg/ml in No. 16. Such a cause may be considered to have relation to a variety of factors. However, a main factor is looked upon as glycerol. Therefore, the content of ethylene glycol in the blood in Fig. 3 shows the increasing amount, that is, the value deducting the level before the injection from that after the injection.

The depressing and ascending curve of ethylene glycol in the blood was appreciably different among each of cases. In general, the content of ethylene glycol in the blood did not nearly change within 1 hour after the injection. But, it increased remarkably from 1 to 2 hours later, reached the maximum at 2 to 3 hours later, and then, decreased immediately.

## DISCUSSION

Up to the present, there have been a lot of literatures as regards the nitroglycol poisoning, whereas the reports concerning its metabolism are very a few so far as the author has carried out an investigation.

Clark et al.[11] separated and identified, together with free nitroglycol, the some intermediatery metabolites, such as ethylene glycol mononitrate, nitrite, and nitrate etc. from the blood of rats which were subcutaneously injected with 65 mg of nitroglycol per kg of the body weight. In addition, Hasegawa and co-workers[9] detected free nitroglycol and a large amount of nitrate in the blood of rabbits receiving 0.2 g of nitroglycol per kg of the body weight. But, they have reported that nitrite was not detected at all at that time.

In the present experiments, free nitroglycol and its metabolites, such as ethylene glycol and nitrate were investigated in the essential point of determination. Therefore, the studies on what kinds of metabolites appear in the blood after the injection of nitroglycol must be entrusted the following experiments. The changes of free nitroglycol and nitrate content in the blood nearly agreed with those of Hasegawa et al[9] Namely, the content of free nitroglycol in the blood increased rapidly after the injection and reached the peak at 1 hour later. Thereafter, it decreased immediately, came to be the trace amount at 6 hours later, and then, vanished completely from the blood at 24 hours later. The content of nitrate in the blood continued to increase during 3 hours after the injection, preserved an almost constant level from 3 till 5 hours later, and then, decreased gradually. In all cases, the content of nitrate in the blood at 24 hours later appreciably exceeded the pre-injection level. The content of ethylene glycol in the blood scarcely varied by 1 hour later. Thereafter, it began to increase rapidly, reached the maximum at 2 to 3 hours later, and then, excluded immediately from the blood.

Judging from the present data, it is considered that the breakdown of nitroglycol takes place as soon as it comes into a body. According to hitherto reports, the hydrolyzing of nitroglycol is considered to be introduced through the non-enzimic and enzymic reactions. In the former case, there is the oxidation of hemoglobin by nitroglycol, viz., the formation reaction of methemoglobin, and the chemical equation has been given as follows by some investigators.[12,13]

$$Hb(O_2) + \begin{vmatrix} CH_2\text{-}O\text{-}NO_2 \\ CH_2\text{-}O\text{-}NO_2 \end{vmatrix} + 3H_2O \rightarrow Hb(OH)_4 + \begin{vmatrix} CH_2\text{-}O\text{-}NO_2 \\ CH_2\text{-}O\text{-}OH \end{vmatrix} + HNO_3$$

In the later case, Clark et al.[11] have suggested that the breakdown of nitroglycol might be carried out by nitroglycerine reductase, inasmuch as nitrite is produced in the course of the metabolism; and they represented the metabolic pathway of nitroglycol by the following scheme.

$$\text{Nitroglycol} \rightarrow \text{Nitrite ester} \begin{cases} \text{Ethylene glycol mononitrate} \\ \text{Nitrite} \end{cases} \rightarrow \text{Nitrate}$$

Provided that nitroglycol is hydrolyzed enzymically, only after it is reduced by reduced glutathione the same as nitroglycerine, it is considered that nitrate may not derived from nitroglycol as having already been pointed out by Heppel and co-workers.[14] However, in the results of Clark et al., nitrite and nitrate, at the same time, appears in the blood of rats after the injection of nitroglycol and a great deal of nitrate is liberated. Therefore, there is a somewhat question for their interpretation that the decomposition of nitroglycol is due to nitroglycerine reductase.

On the other hand, Hasegawa et al.[12] discovered that nitrate is produced when nitroglycol is incubated with the blood and they have suggested that "nitroglycol hydrolylase", a specitic enzyme acting on nitroglycol exists in the blood. One is distributed in the liver, besides the blood and hydrolyze nitroglycol into nitrate and alcohol. Moreover, it is said that the enzyme in the erythrocytes has an optimum activity at PH 6.5 and need not the reduced form of glutathione as a prosthetic group in the action, and its Michaelis constant are 3 mM at 37°C. Therefore, nitroglycol hydrolylase obviously differs from nitroglycerine reductase and there is a discrepancy between both investigators in the point of a kind of enzyme concerning with nitroglycol and of the mechanism of action. At the present, the author has not any data available for analyzing their results. Whereas, the author has a great interest on this problem as a subject for studies in the future.

## II. The Formation of Methemoglobin and Its Metabolism

### INTRODUCTION

As the well-known findings in the nitroglycol poisoning, there are the formation of methemoglobin, the appearance of heinz bodies, anemia, and the decrease in the activities of enzymes existed in the erythrocytes. In especial, the formation of methemoglobin and the appearance of heinz bodies are looked upon as the most remarkable findings. From such a reason, Gross[15] has advocated taking a good use of methemoglobin or heinz bodies for an evidence of the nitroglycol poisoning. Whereas, the studies concerning the mechanism of methemoglobin formation have been as yet only a few. Therefore, the author has carried out further some studies on the formation of methemoglobin and its mechanism.

### MATERIALS AND METHODS

Animals: Healthy, male rabbits weighing from 2.0 to 3.0 kg were used and

fed on solid rabbit feed.

Materials : Ten % nitroglycol olive solution (Nipponkayaku Co.), olive oil registered as Ⓕ, 10 % nitroglycerine olive solution (Nipponkayaku Co.), sodium nitrite, sodium nitrate, and potassium ferricyanide.

Methods of administration of nitroglycol : a) The rabbits used for the determination of methemoglobin were treated as follows. The poisoned rabbits were subcutaneously injected with 0.15 to 3.0 g of nitroglycol per kg of the body weight and the controls subcutaneously received 1.5 to 3.0ml of olive oil per kg of the body weight. b) The rabbits available for the measurment of absorption spectrum of methemoglobin were treated as follows. In a case of nitroglycerine, the rabbits were treated in the same as them poisoned by nitroglycol. In another case, the rabbits subcutaneously received the suitable amounts of the aqueous solutions of some reagents, respectiviely.

Blood sampling : The blood was punctured from the heart with an injection syringe and rapidly transferred into a test tube containing heparin.

Determination of methemoglobin[16] : One half ml of the blood was diluted with 25 ml of M/60 phosphate buffer (PH 6.5). After hemolyzed by adding several milligrams of saponin, the diluted blood solution was mixed well and divided into two equal portions. The optical density of one of the portions was measured against the phosphate buffer at the wavelength of 635 mμ (E'MHb). This aliquot was returned to its respective tube and to this, was added one drop of 10 % sodium cyanide solution (freshly neutrized with an equal volume of 12 % acetic acid). The solution was shaked well and its optical density was measured (E'HbCN). Subsequently, to the second portion of the blood sample was added one drop of 5% potassium ferricyanide solution. The solution was mixed well and its optical density was measured (EMHb). This aliquot was returned to its respective tube and to this, was added one drop of 10 % sodium cyanide solution. The solution was mixed well and its optical density was measured (EHbCN). The percentage of methemoglobin to total hemoglobin was calculated according to the following formula.

$$\frac{\text{E'MHb–E'HbCN}}{\text{EMHb–EHbCN}} \times 100 = \%\ \text{Methemoglobin}$$

Measurment of absorption spectrum of methemoglobin : In the in vivo experiments, the blood was punctured from the heart at 2 hours after the injection and diluted to 50 times with 0.01 M phosphate buffer (PH 6.5) prior to the measurment. In the in vitro experiments, the blood first was diluted to 50 times with 0.01 M phosphate buffer (PH 6.5), followed by adding the suitable amount of each of some reagents.

## RESULTS

The hourly change of methemoglobin content in the blood:

The rabbits were subcutaneously injected with 0.15 to 3.0 g of nitroglycol per kg of the body weight and the content of methemoglobin in the blood was recorded with the lapse of time (Table 1).

Table 1. The Percentage of methemoglobin to total hemoglobin after the injection of nitroglycol.

| Time (hr.) / Rabbit No. | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 17 | 1.64 | 37.97 | 46.57 | 38.06 | 18.06 | 12.56 | 3.84 | 3.92 | 2.09 |
| 18 | 0.47 | 9.19 | 41.19 | 47.85 | 35.69 | 33.69 | 19.82 | 10.54 | 5.88 |
| 19 | 0.68 | 22.72 | 22.50 | 12.50 | 4.25 | 2.16 | 3.02 | 0.88 | 0.30 |
| 20 | 0.21 | 18.41 | 27.08 | 25.76 | 14.32 | 8.90 | 1.18 | 1.20 | 0 |

Methemoglobin was formed immediately after the injection of nitroglycol and reached the maximum already at 1 to 3 hours later. Thereafter, it began to decrease, fell into the trace amounts at 8 hours later, and then, could not be detected in the blood at 24 hours later in all cases. On the other hand, the forming rate of methemoglobin corresponded to about 2 to 3 times of its diminishing rate, and besides, the forming amount of methemoglobin was approximately proportional to the amount of nitroglycol administrated.

The daily change of methemoglobin content in the blood:

The rabbits were subcutaneously injected 0.15 to 3.0 g of nitroglycol per kg of the body weight every day and the content of methemoglobin in the blood was plotted with the lapse of day (Fig. 4 and 5). Moreover, in order to obtain a clue for "Monday disease", the symptom characteristic for nitroglycol poisoning, the injection was stopped for 2 days during this experimental period.

The content of methemoglobin in the blood at the maximum increased from first day to second day. But, after third day, it decreased gradually. The forming rate of methemoglobin scarcely varied during a test term, while its diminishing rate decreased daily. In especial, the lowering of diminishing rate was distinctly observed after two offdays. In the cases received only olive oil, no methemoglobin was formed.

The absorption spectrum of methemoglobin:

In order to clarify whether or not methemoglobin is produced by nitroglycol itself, the absorption spectrum of methemoglobin formed after the treatment with nitroglycol was studied in comparison with those formed after the treatment with the other reagents.

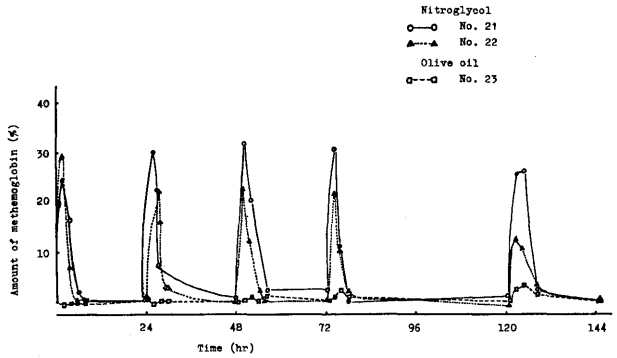



Fig. 4. The change of percentage of methemoglobin to total hemoglobin after the injection of nitroglycol.

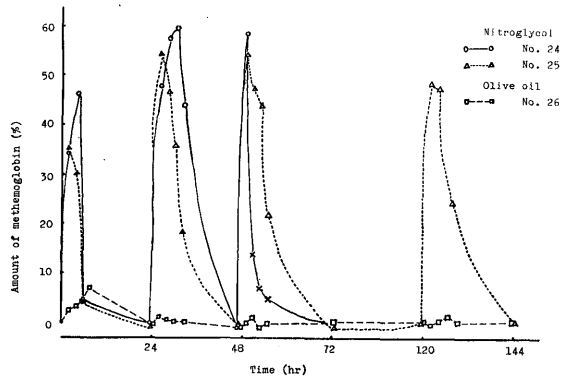



Fig. 5. The change of methemoglobin to total hemoglbin after the injection of nitroglcyol.

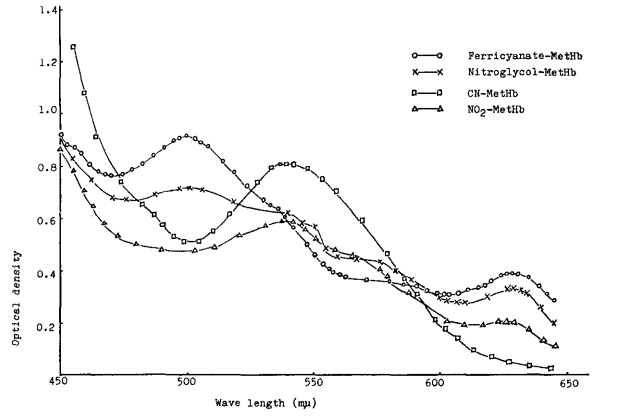



Fig. 6. Absorption spectra of methemoglobin formed in vitro by different reagents.

As the in vitro results are shown in Fig. 6, the absorption spectrum of methemoglobin formed after the addition of nitroglycol was in accord with that formed after the addition of potassium ferricyanide, that is, both methemoglobin each showed the absorption maxima at 500 and 630 mμ. On the contrary, the methemoglobin formed after the addition of sodium nitrite had the absorption maxima at 540 and 623 mμ and was differenciated from the formers. In the blood treated with sodium nitrate, no methemoglobin was formed and all types of methemoglobin were converted into CN-methemoglobin (having single absorption at 540 mμ) by addition of sodium cyanide.

As the in vivo results are illustrated in Fig. 7, the absorption spectrum of methemoglobin formed after the administration of nitroglycol closely agreed with that in vitro and showed the maxima at 500 and 630 mμ. Whereas, the absorption spectrum formed after the administration of sodium nitrite was different from that in vitro and had the maxima at 500 and 630 mμ. Moreover, in the bloods of rabbits administrated sodium ferricyanide and sodium nitrate, no methemoglobin was formed.

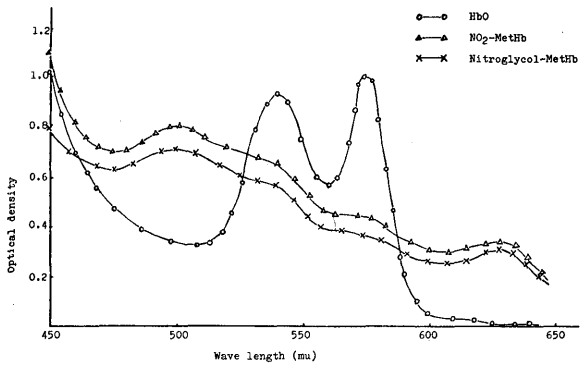



Fig. 7. Absorption spectra of methemoglobin formed in vivo by nitrite nitroglycol and oxyhemoglobin.

## DISCUSSION

The fact that the considerable amounts of methemoglobin and heinz bodies are formed by the action of nitroglycol has already been well-known. Symanski[7] has reported that 70, 80, and 90 percent methemoglobin each was detected in the blood of three cases who died of the nitroglycol poisoning. However, the evidence of methemoglobin for the fatal cases by the nitroglycol poisoning and for the workers engaged in the explosive plants is said generally to be impossible. As such the reasons, it is thought that methemoglobin can not be detected already in the time the test is carried out by the reason of its rapid disappearance ; the amount of nitroglycol by which the workers are exposed in the plant come to decrease

conspicuously ; and the repeated exposure of nitroglycol resulted in the development of tolerance of erythrocytes to nitroglycol etc. On the contrary, in the experiments using animals, it has already been demonstrated by many investigators that methemoglobin was formed easily and in large quantities by nitroglycol. Katsunuma et al.[17] administrated 0.1 to 0.25 g of nitroglycol per kg of the body weight to cats and detected 60 to 80 percent methemoglobin. Suwa et al.[5] injected 0.3 to 0.4 g of nitroglycol per kg of the body weight to rabbits and found 50 to 60 percent methemoglobin.

In the present experiments, the change of methemoglobin content after the injection of nitroglycol was observed hourly and daily. These results were approximately in accord with Abe et al.[18] and Hasegawa et al.[19], respectively. In the observation within 24 hours after the injection, methemoglobin was formed rapidly after the injection and reached the maximum at 1 to 3 hours later. The content of methemoglobin at the maximum each was 23 to 27 percent in the cases received 0.15 g of nitroglycol per kg of the body weight and 45 to 50 percent in the cases injected with 0.3g of nitroglycol as the same. On the other hand, the disappearance of methemoglobin from the blood occurred rapidly and at 24 hours later, no methemoglobin could be detected. In the case of successive observation during 4 days, the content of methemoglobin at the maximum increased from first to second day, but third day later decreased gradually. Such a tendency may be considered to be based on the development of tolerance of erythrocytes to nitroglycol. The forming rate of methemoglobin was maintained in the about constant value during this experimental period, while its diminishing rate gradually decreased. In especial, the lowering of forming rate was noticed after two offdays and such a inclination may concern with "Monday disease", viz., a specific symptom of nitroglycol.

A variety of chemicals are capable of inducing the formation of methemoglobin, either by a direct stoichiometric action in which one mole of the hemoglobin to form one mole of methemoglobin, or by metabolic transformation to derivative which directly acts on the hemoglobin.[20][21] For examples, in the case of phenylhydroxylamine, it reacts with oxyhemoglobin to form nitrosobenzene complexed with hemoglobin and hydrogen peroxide, the latter which is unstable and yields methemoglobin. The nitrosobenzene is in turn reduced by the enzyme, diaphorase, resulting in the reformation of phenylhydroxylamine. Thus, one molecule of phenylhydroxylamine can result in the formation of several molecules of methemoglobin.

Therefore, in order to whether or not the methemoglobin formed after the administration of nitroglycol is produced either by nitroglycol itself or its intermediate metabolites, the absorption spectrum of methemoglobin formed after the treatment of nitroglycol was in comparison with those formed after the treatment of different reagents. The absorption spectrum of methemoglobin formed in vitro

by nitroglycol showed its absorption maxima at 500 and 630 mμ and closely agreed that formed in vivo. Besides, the absorption spectrum was in accord with that formed in vitro by potassium ferricyanide. On the other hand, the absorption spectrum of methemoglobin formed by sodium nitrite differed between the in vitro and in vivo types, that is, the former showed the absorption maxima at 540 and 623 mμ and the latter showed the absorption maxima at 500 and 630 mμ. Moreover, in the in vivo case for potassium ferricyanide and both the in vitro and in vivo cases for sodium nitrate, methemoglobin each was not produced.

There are nitrate and nitrite as the intermediate metabolites of nitroglycol. Out of them, nitrate, itself is not capable of inducing the formation of methemoglobin except the biotransformation[22] of it into nitrite through the reducing action of bacilli in the intestine.

On the contrary, nitrite converts oxyhemoglobin into methemoglobin, while the absorption of methemoglobin is different from that formed by nitroglycol. Besides these, taking the correlation between the content of methemoglobin and the amount of free nitroglycol at the maximum in the blood and furthermore, the rapidity in the formation of methemoglobin by nitroglycol into consideration, the formation of methemoglobin after the administration of nitroglycol may be considered to attribute to the direct action of nitroglycol itself rather than its metabolites and such an assumption well agrees with the theoretical considerations concerning the oxidation of hemoglobin to methemoglobin by nitroglycol provided by Hasegawa et al.[12] and Kakizaki et al.[13]

## III. The Variation of Serum Transaminase and Catalase Activities

### INTRODUCTION

Nitroglycol has already been known to behavior to monoamine oxidase[23] and alcohol dehydrogenase[24] as their inhibitors. Both the enzymes are noticed in the respect having SH-group at their active sites.

On the other hand, there is cardiac crises occurred often holidays or its next holidays as one of the characteristic symptoms of the nitroglycol poisoning. In accordance with Kubota,[25] the pathological changes seem to be observed in each organ of animals administrated of nitroglycol for a long term.

From such the findings, it is considered that nitroglycol may give rise to the increase of activity in serum glutamic-oxalacetic transaminase (SGO-T) and serum glutamic-pyruvic transaminase (SGP-T) and the inhibition of activity in catalase. Therefore, the author administrated nitroglycol to the rabbits and observed the change of these enzymic activities with the lapse of time.

## MATERIALS AND METHODS

Animals: Healthy, male rabbits weighing from 2.0 to 3.0kg were used and fed on solid feed. Only the rabbits available for the assay for SGO-T and SGP-T activities did not feed during 12 hours prior to the measurment.

Materials: Ten % nitroglycol olive solution (Nipponkayaku Co.), olive oil registered as Ⓕ.

Methods of administration: The poisoned rabbits were subcutaneously injected with 0.15 to 0.2g of nitroglycol per kg of the body weight and the controls were subcutaneously received 0.15 to 2.0 ml of olive oil per kg of the body weight.

Blood sampling: The blood was punctured from the heart with an injection syringe and transferred into the test tube containing heparin.

Assay of SGO-T and SGP-T activities: Substrate solution for SGO-T (2660mg % l-aspartic acid, 29.2 mg $\alpha$-ketoglutaric acid), substrate solution for SGP-T (1780 mg % dl-alanine, 29.2mg % $\alpha$-ketoglutaric acid), 4 N sodium hydroxide, standard solution for caliburation curve (18.8mg % lithium pyruvate). All the reagents were punctured as a kit manufactured by Yatron Co. based upon Reitman-Frankel's method.

The blood was centrifuged for 5 minutes 3,000 r.p.m. and the serum was separated out. The hemolized blood was eliminated from the assays for these transaminases because it gives higher activities as usual. One ml of the substrate solution for SGO-T (or SGP-T) was transferred into a test tube and incubated for 5 minutes at 37°C. To this, was added 0.2 ml of the serum and the mixture was shaked rigorously. It was incubated for 60 minutes in SGO-T and 30 minutes in SGP-T. After the incubation, 1 ml of 2,4-dinitrophenylhydrazine was added and the mixture was stood for 20 minutes at room temperature. Subsequently, 10 ml of 0.4 N sodium hydroxide (4 N sodium hydroxide was diluted 10 fold with pure water as needed) was added and the mixture was stood for 10 minutes at room temperature. The optical density was measured against pure water at the wavelength of 505 m$\mu$. Both activities were calculated from the caliburation curve using lithium pyruvate and the units of activities were expressed as Karmen's unit.

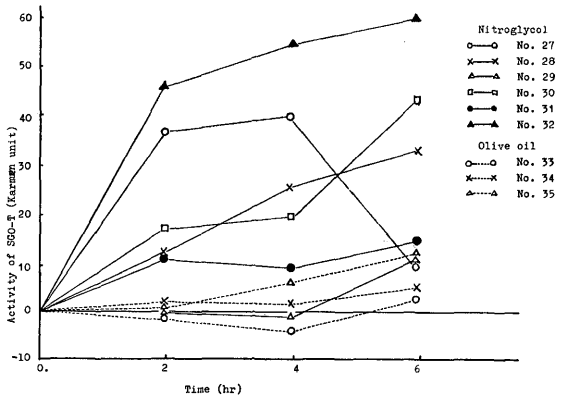
Assay for catalase activity[19]: 0.01 M hydrogen peroxide, 0.1 M hydrogen peroxide, 0.1 M phosphate buffer, 2 N sulfuric acid, 0.005 N potassium permangenate. The diluted blood solution (0.1 ml of the blood was diluted with 3 ml of pure water), 2ml of 0.1 M phosphate buffer and 4ml of pure water were transferred into a conical flask in that order and the flask was immersed for 5 minutes into ice water at 0°C. After the pretreatment for 3 minutes by addition of 1 ml of 0.01 M hydrogen peroxide, 2 1ml of 0.1 M hydrogen peroxide was added and the catalase reaction was made to proceed for 15 seconds. Subsequently, 5ml of the solution was promptly sucked up, followed by blowing the conical flask

containing sulfuric acid. The remained hydrogen peroxide was titrated with 0.005 N potassium permangenate and the activity of the blood catalase was defined to be 100 percent when the rate of catalase reaction corresponded to 250 $\mu$M per second.

## RESULTS

The change in the activities of SGO-T and SGP-T

The poisoned rabbits were subcutaneously injected with 0.2g of nitroglycol per kg of the body weight and the controls were received 2 ml of olive oil per kg of the body weight. The activities of both transaminases in their sera were assayed at an interval of 2 hours for 6 hours after the injection.




Fg.i 8. The change of serum glutamic-oxalacetic transaminase (SGO-T) after the injection of nitroglycol.

The depressing and ascending curve of SGO-T activity was the significant difference between nitroglycol and control groups (Fig. 8). Namely, the activity in the former brought about the appreciable increase already at 2 hours later and its elevating rate of each rabbit at that time was as follows: 2.5 in No. 27, 1.4 in No. 28, 1.0 in No. 29, 1.9 in No. 30, 1.3 in No. 31, and 4.8 in No. 32. Thereafter, it kept further increasing gradually. On the contrary, the activity of SGO-T in the latter did not nearly changed with the lapsed time.

On the other hand, the activity of SGP-T in the nitroglycol group scarcely elevated and its depressing and ascending curve was similar to that in the control group (Fig. 9). The activities of both transaminases before the injection were $26.3\pm7.6$ (9 rabbits) for SGO-T and $22.0\pm5.9$ (8 rabbits) for SGP-T.

The change in the activity of catalase:

The poisoned rabbits were subcutaneously injected with 0.15 g of nitroglycol per kg of the body weight and the controls received 1.5 ml of olive oil per kg of the body weight. The catalase activity in the blood was plotted against time after the injection.

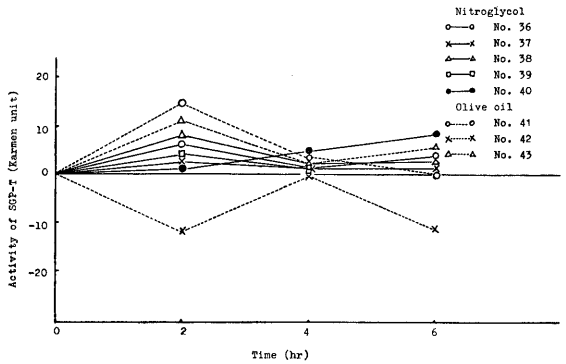



Fig. 9. The change of serum glutamic-pyruvic transaminase (SGP-T) after the injection of nitroglycol.

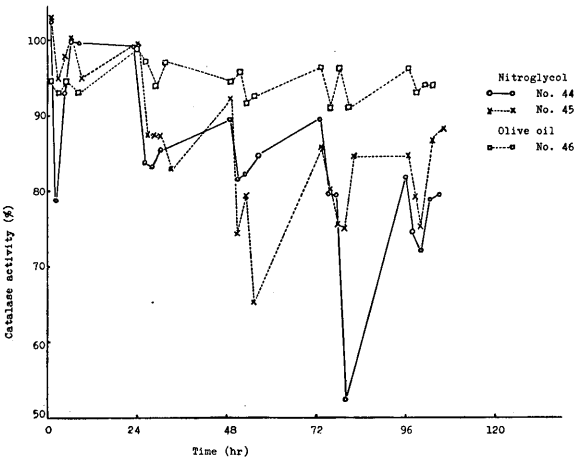



Fig. 10. The change of catalase activity in the blood after the injection of nitroglycol.

As seen in Fig. 10, the catalase activity was clarified to be inhibited by nitroglycol. Namely, the inhibition of catalase activity appeared somewhat immediately after the injection and reached the maximum at 6 hours later. Thereafter, it became weak gradually, while the catalase activity did not return to the pre-injection level even at 24 hours later. The catalase activity at 24 hours later lowered slowly through the repetition of administration. On the other hand, the catalase activity in the control group slightly decreased after the injection, whereas it returned to the pre-injection level already at 24 hours later. Besides, the catalase activity at 24 hours later remained in the approximately constant level.

## DISCUSSION

GO-T and GP-T are widely distributed in human organs. The former is most concentrated in the heart muscle, followed by the liver, skeletal muscle, kidney, pancreas, and serum in decreasing order.[26] On the other hand, the latter is concentrated in the liver, followed by the kidney, heart muscle, skeletal muscle, and serum in decreasing order. Thus, the distribution amount of GO-T and GP-T in the normal serum is very little. On the contrary, if a variety of damages are suffered on different organs, these enzymes transfer from the corresponding organ into serum and as the result, the significant elevation of SGO-T and SGP-T activities occurs.

Since LaDue and co-workers[27] have reported the elevation of SGO-T activity in the patients with myocardial infarction,[26] a lot of investigations[26)28] have been undertaken on the relationship between the damaged organs and the variation of the activities of serum transaminases. In addition, the elevation of SGO-T and SGP-T activities after the administration of drugs and poisons has also demonstrated by many investigators. For example, there are reports bring about the significant increase of both SGO-T and SGP-T activities in the dosage of some chemicals, such as aspirin,[29] salicylic acid,[29] bishydroxycoumarin (dicumarol)[30], and carbon tetrachloride;[31)32] and the report concerning the elevation of SGO-T activity after the administration of iproniazid[33] (marsilid) and alcohol to the patients of alcoholism.[34] In general, it seems that SGP-T activity alters more easily than SGO-T activity in the administration of drugs and poisons.[30] Such a cause is looked upon as the liberation of transaminases from the liver.[29)32]

In the present experiments, the activity of SGO-T showed the appreciable increase even at 2 hours after the injection of nitroglycol and thereafter, continued to increase gradually. On the contrary, the activity of SGP-T hardly varied with the lapsed time. From these findings, it is assumed that nitroglycol attacks the heart muscle to give rise to any damage. And such a cause inducing the elevation of SGO-T activity may be considered to be responsible for the results that nitroglycol brought about the abnormal metabolism, the disturbance of acid-base equilibrium in the blood, the damages of blood vessels, and the degeneration and necrosis of cells.

Catalase is one of the enzymes by which have been well-known before. The activity has also been reported to be inhibited by different inorganic substances, peroxides, and pigment,[35] e.g., KCN, NaF, NO, $NaN_3$, $NH_3$, $NH_2OH$, $NH_2NH_2$, $CH_3COOH$, $C_2H_5OOH$, 2,4-dinitrophenol, and thioaniline. Therefore, nitroglycol behaving as an oxidative agent is presumed to give any effect on the activity of catalase. Whereas, the report on this subject is nothing except an instance up to the present. Hasegawa and co-workers[19] have comfirmed the fact that nitroglycol inhibits the activity of catalase and provided the following equations for the

reaction of inhibition. $E+S \rightleftarrows ES$, $ES+S \rightleftarrows ESS$, $ESS \rightarrow E+$product. Furthermore, they postulated that ESG and EG complexs are produced in the course of the reaction between nitroglycol and catalase, and in general, the dissociation constant of the former exceeds that of the latter so that the reaction curve without pretreatment by hydrogen peroxide bends with time corresponding to the reaction. However, there is not clarified in the respect of "what a substance acting as an inhibitor of the catalase reaction is".

In this experiment, it was elucidated the fact that the activity of catalase was inhibited by nitroglycol; and the result was in accord with that of Hasegawa et al. The inhibition of catalase activity appeared more rapidly after the injection of nitroglycol and reached the maximum about at 6 hours later. Thereafter, it began to lower gradually, but the activity of catalase did not restore to the pre-injection level even at 24 hours later. In addition, the activity of catalase at 24 hours later decreased gradually along with the times of injection.

## IV The Change of Reduced Glutathione, Lactic Acid, and Blood Sugar Content in the Blood

### INTRODUCTION

There exist Embden-Meyerhof pathway (E-M pathway) and Warburg-Dickens-Lipman pathway (W-D-L pathway) in the blood. Out of them, the former furnishes all energies necessary for the living of erythrocytes and the latter plays an important part to the protection of hemoglobin from the formation of methemoglobin. On the other hand, it is considered that nitroglycol prevents both pathways from their smooth proceeding, because it behaves oxidatively to erythrocytes and forms a great amount of methemoglobin and heinz bodies in erythrocytes.

Therefore, the author administrated a definite amount of nitroglycol to the rabbits and observed the changes of some substances related to these pathways with the lapsed time.

### MATERIALS AND METHODS

Animals: Healthy, male rabbits weighing from 2.0 to 3.0 kg were used and fed on solid rabbit feed.

Materials: Ten % nitroglycol olive solution (Nipponkayaku Co.), olive oil registered as Ⓕ.

Methods of administration: The poisoned rabbits were subcutaneously injected

with 0.2 g of nitroglycol per kg of the body weight and the controls were subcutaneously received 2.0 ml of olive oil per kg of the body weight.

Blood sampling: The blood was taken cutting off the veins of ears. Sodium oxalate for the blood of reduced glutathione and heparin for the other bloods, respectively, were used as anticoagulants.

Determination of reduced glutathione in the blood[36]: Twenty-five % metaphosphoric acid, 5 % metaphosphoric acid, 0.1 M alloxan, 0.5 N sodium hydroxide (10 ml of this solution is equivalent to a mixture of 10 ml of 5 % metaphosphoric acid and 10 ml of 0.1 M alloxan. The mixture was adjusted to PH 7.5 with sodium hydroxide as needed), 0.5 M phosphate buffer, 1 N sodium hydroxide, reduced glutathione standard solution (25.0mg of reduced glutathione was dissolved in 500ml of 5% metaphosphoric acid).

One half ml of oxalated whole blood was transferred into a centrifugal tube and then, 3.5 ml of water was added. The mixture was shaked well and allowed to stand for 5 minutes at room temperature in order to be completely hemolyzed. Subsequently, 1.0ml of 25 % metaphosphoric acid was added and centrifuged for 5 minutes at 7000 r.p.m. (0°C). The supernatant was prepared as follows.

b = 1.0 ml of 5 % metaphosphoric acid + 1.0ml of 0.1 M alloxan

$b_0$ = 1.0 ml of 5 % metaphosphoric acid + 1.0 ml of pure water

s = 1.0 ml of reduced glutathione solution + 1.0 ml of 0.1 M alloxan

$s_0$ = 1.0 ml of reduced glutathione solution + 1.0 ml of pure water

x = 1.0 ml of supernatant + 1.0 ml of 0.1 M alloxan

$x_0$ = 1.0 ml of supernatant + 1.0 ml of pure water

First, into b of the test tubes, each 1 ml of 0.5 M phosphate buffer and 0.5 N sodium hydroxide was added and the mixture was shaked well. This procedure was repeated with each successive tube at 30 seconds intervals. After the lapse of 6 minutes exactly, 1.0 ml of 1 N sodium hydroxide was added to each tube at 30 seconds intervals in order to prevent the reaction from further proceeding. The optical density of b,s, and x solutions was measured, respectively, against $b_0$, $s_0$, and $x_0$ solutions at the wavelength of 305 m$\mu$ and the content of reduced glutathione per 100 ml of the blood was calculated according to the following equation.

$$\text{mg Reduced glutathione/100 ml} = \frac{Ax-Ab}{As-Ab} \times 0.05 \times \frac{100}{0.1} = \frac{Ax-Ab}{As-Ab} \times 50$$

Determination of lactic acid in the blood[37]: Ten % zinc sulfate solution, 0.5 N sodium hydroxide, 20 % copper sulfate solution, 4 % copper sulfate solution, powdered calcium hydroxide, p-hydroxydiphenyl reagent (1.5g of p-hydroxydiphenyl was dissolved into 10 ml of 5 % sodium hydroxide plus little pure water by warming and diluted to 100ml with pure water), concentrated sulfuric acid, lactic acid standard solution (21.3g of lithium lactate was dissolved into a small amount of water and then, 0.5ml of concentrated sulfuric acid was added. The volume

was adjusted to 100 ml with pure water).

Whole blood, water, and lactic acid standard solution each was treated as follows. In a centrifugal tube containing 0.5 ml of either of the samples, 3.5 ml of pure water added and allowed to stand at room temperature. After 5 minutes, to this was added each 0.5 ml of 10 % zinc sulfate and 0.5 N sodium hydroxide. The mouth of centrifugal tube was covered with parafilm and the mixture was shaked rigorously, followed by centrifuging for 10 minutes at 7000 r.p.m. (0°C). One ml aliquot of the supernatant was transferred into a test tube and to this were added 0.5 ml of 20% copper sulfate solution, 3.5 ml of pure water, and 0.5g of powered calcium hydroxide, in that order. The mouth of test tube was covered with parafilm and the mixture was shaked rigorously until solids uniformly disperse. After allowed to stand for 15 minutes at room temperature, the mixture was repeated shaking again, and allowed to stand another 15 minutes. Subsequently, it was centrifuged for 10 minutes at 7000 r.p.m. (0°C). One ml aliquot of the supernatant was transferred into a test tube and to this was added one drop of 4% copper sulfate solution. The test tube thus treated was immersed into a ice water bath, followed by adding 6 ml of previously cooled concentrated sulfuric acid carefully and very slowly (the temperature of mixture was especially noticed not to exceed 10°C). It was heated in a boiling water bath for 5 minutes and transferred into running tap water. After cooling, 0.1ml of p-hydroxydiphenyl reagent was added and the mixture was shaked well. Subsequently, the test tube was left in an incubator for 1 hour at 25°C with occasional mixing. Again, it was heated in the boiling water bath for 90 seconds to destroy excess reagent and then, was cooled under running tap water. The optical density was measured against pure water at the wavelength of 570 m$\mu$ and the amount of lactic acid per 100ml of the blood was calculated on the basis of the following equation.

$$\text{mg Lactic acid}/100\,\text{ml} = \frac{Ax-Ab}{As-Ab} \times 0.004 \times \frac{100}{0.02} = \frac{Ax-Ab}{As-Ab} \times 20$$

Determination of blood sugar[38]: Copper reagent A (25 g of sodium carbonate, 25 g of Rochelle salt, 20 g of sodium bicarbonate, and 200 g of sodium sulfate each was dissolved into 800 ml of pure water and the volume was diluted to 1000 ml. The solution was stored below 20°C and filtered after several days), copper reagent B (15g of copper sulfate was dissolved into 100ml of pure water containing one drop of concentrated sulfuric acid), arsenomolybdate color reagent (25g of ammonium molybdate was dissolved into 450ml of pure water, to this were added 21 ml of concentrated sulfuric acid and 3 g of $NaHAsO_4 \cdot 7H_2O$ dissolved previously in 25 ml of pure water, and the mixture was shaked rigorously. This solution was used after it was placed in an incubator at 37°C for 2 days), 0.3 N barium hydroxide (47 g of barium hydroxide first was added into the small amount of boiling water, dissolved completely by further boiling for 2 to 3 minutes, and the mixture was adjusted to 100 ml with pure water. The solution was stored

in a bottle protected by soda lime from carbon dioxide), 5 % zinc sulfate solution, glucose standard solution (the range of concentrations was prepared to 100, 200, 300, and 400 mg per 100ml).

To a centrifugal tube containing 1.5 ml of pure water and 0.1 ml of the blood was added each 0.2 ml of 5 % zinc sulfate solution and 0.3 N barium hydroxide and the centrifugal tube was shaked rigorously covering its mouth with parafilm. The solution was allowed to stand for 5 minutes at room temperature and centrifuged for 10 minutes at 7000 r.p.m. (0°C). One ml aliquot of the supernatant was transferred into a 25 ml-flask, to this was added 1.0 ml of a mixture copper reagent A and B (25 parts of reagent A and 1 Part of reagent B were mixed well as needed), and the mixture was shaked rigorously. Subsequently, the solution was heated in a boiling water bath for 20 minutes and transferred into running tap water. After cooling, 1.0 ml of arsenomolybdate color reagent was added, carbon dioxide was evoluted utterly with occasional shaking, and then, the volume was adjusted to 25 ml with pure water. The optical density was measured against the reagent blank at the wavelength of 520 mμ and the amount of blood sugar per 100 ml of the blood was calculated from the calibration curve produced previously.

## RESULTS

The change of reduced glutathione content in the blood:

The poisoned rabbits were subcutaneously injected with 0.2g of nitroglycol per kg of the body weight and the controls received 2ml of olive oil per kg of the body weight and the change of reduced glutathione content in the blood was observed with the lapsed time.

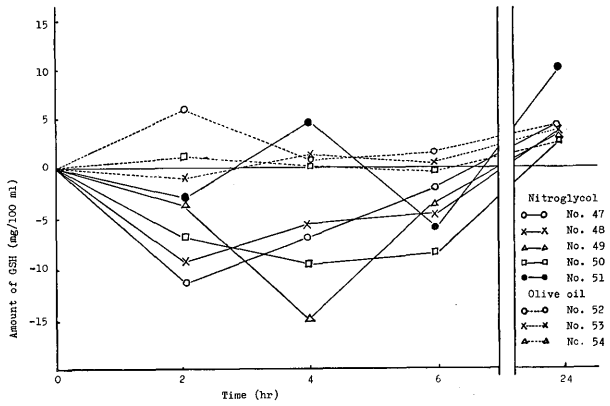



Fig. 11. The change of reduced glutathione (GSH) in the blood after the injection of nitroglycol.

As seen in Fig. 11, the decreasing amount of reduced glutathione in the nitroglycol group was very small, but its depressing and ascending curve was distinctly differenciated from that in the control group. Namely, the content of reduced glutathione in the former decreased immediately after the injection and reached the minimum 2 to 4 hours later. Thereafter, it began to increase and at 24 hours later, slightly exceeded the pre-injection level in all cases. On the other hand, the content of reduced glutathione in the latter was kept about constant during 24 hours except a value of No. 52 at 2 hours later and its depressing and ascending curve was flatness approximately. In addition, the concentration of reduced glutathione before the injection (8 cases) was 41.4±4.16mg per 100ml of the blood.

The change of lactic acid content in the blood:

The change of lactic acid content in the blood was observed with the lapsed time after the injection of nitroglycol as treated the same as reduced glutathione.

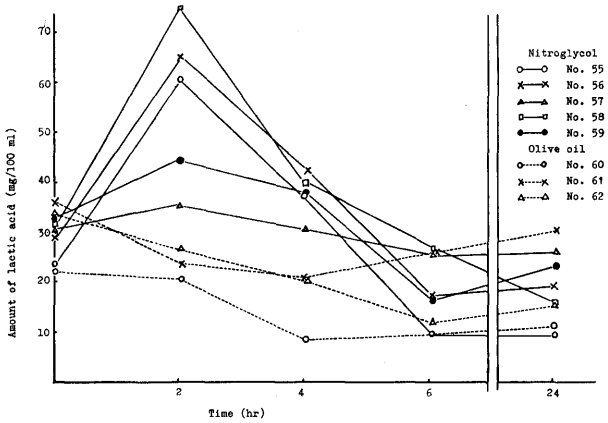



Fig. 12. The change of lactic acid in the blood after the injection of nitroglycol.

As shown in Fig. 12, the depressing and ascending curve of lactic acid content in the nitroglycol group was different from that in the control group. Namely, the content of the lactic acid in the former reached the maximum already at 2 hours after the injection and at that time, its increasing rate was 2.32 in No. 55, 2.38 in No. 56, 1.13 in No. 57, 2.36 in No. 58, and 1.34 in No. 59. Thereafter, it decreased rapidly and reached the minimum in 4 cases out of 5 cases at 6 hours later. Again, it began to increase, but did not restore to the pre-injection level even at 24 hours later. On the other hand, the content of lactic acid in the latter decreased slightly over 4 to 6 hours, but at once, it recovered. And its content at 24 hours later was little below than the pre-injection level as well as the nitroglycol group. In addition, the concentration of the lactic acid before the injection (8 cases) 30.21±4.54mg per 100ml of the blood.

The change of blood sugar:

The rabbits were treated as well as the cases of reduced glutathione and lactic acid, and the content of blood sugar was measured using the blood taken at 1, 2, 4, 6, and 24 hours after the injection.

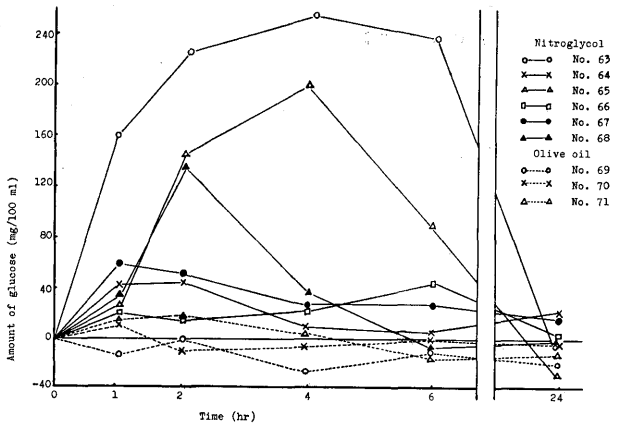



Fig. 13. The change of sugar in the blood after the injection of nittroglycol.

As shown in Fig. 13, the hourly change of blood sugar content was recognized of the significant differences between the nitroglycol and corresponding control groups. The depressing and ascending curve of blood sugar in the former was difficult to be summarily elucidate because the individual variation appeared strongly. In general, the content of blood sugar increased rapidly after the injection and reached the maximum at 2 to 4 hours later. The increasing rate at the peak was as follows; 4.81 in No. 63, 1.55 in No. 64, 3.39 in No. 65, 1.63 in No. 66, 1.71 in No. 67, and 2.57 in No. 68. In especial, there is noticed on the increase of cases of Nos. 63, 65, and 68, and out of them, the concentration of blood sugar in the first case was 318 mg per 100 ml of the blood. Thereafter, it began to gradually decrease and 24 hours later, returned to the pre-injection level or slightly below. On the other hand, the content of blood sugar in the control group did not nearly increase or decrease. In addition, the concentration of blood sugar before the injection (9 cases) was $79.4 \pm 10.47$ per 100 ml of the blood.

## DISCUSSION

The amount of reduced glutathione in erythrocytes occupies about 90 percent of another SH-group substance with the exception of protein. Most of glutathione exists in the reduced form and plays an important role on the oxidation and reduction in cells. In addition, reduced glutathione has been known to carry out the conversion of methemoglobin into oxyhemoglobin as well as ascorbic acid[39] and such a reduction seems to be performed throughout W-D-L pathway.

Therefore, provided that nitroglycol give any effect on the substances related to W-D-L pathway, it is considered that the decrease of reduced glutathione in erythrocytes results.

Yamamoto and co-workers[40] have reported that there were no significant differences in the amount of reduced glutathione between the workers suspected of the nitroglycol poisoning and healthy those in a certain explosive plant. Such a cause may be considered due to either the reasons of which, nitroglycol amount that the workers were exposed in the explosive plant was very small or erythrocytes possesed an tolerance in the result of successive exposure to nitroglycol.

In the present experiments, reduced glutathione in the blood did not particularly decrease, but its depressing and ascending curve in the nitroglycol group was significantly different from that in the corresponding control group. Namely, the content of reduced glutathione decreased rapidly after the injection and reached the minimum at 2 to 4 hours later and then, increased gradually. On the other hand, the content of reduced glutathione in the latter did not nearly vary with the lapse of time.

The cause inducing the lowering of reduced glutathione in the nitroglycol group may be considered as follows with reference to some literatures. Fraser[41] has reported that the formation of methemoglobin and the lowering of reduced glutathione were brought about by hydroxyl types of primaquinine and acetanilid and such the phenomena appeared strongly in glucose-6-phosphate dehydrogenase-deficient erythrocytes. According to the report of Beutler and co-workers,[42] it is said that there is a marked drop in the content of reduced glutathione when acetylphenylhydrazine is incubated with primaquinine sensitive red cells, while there is no effects on reduced glutathione in the incubation with non-sensitive red cells. In addition, Scheiderat-Shahab[43] have described that in the cases administrated of phenylhydroxyamine, nitrobenzene, and aniline, the formation of heinz bodies and the decrease of reduced glutathione each was observed, but in a case received nitrite, thus changes did not occur in spite of the formation of a great deal of methemoglobin. Under the consideration of these reports, the decrease of reduced glutathione content seems to be remarkable when glucose-6-phosphate dehydrogenase in erythrocytes are inhibited or deficient. Moreover, reduced glutathione is likely to be more closely related to the appearance of heinz bodies than the formation of methemoglobin; and such a reason may be presumed on the basis of SH-group. On the other hand, the depressing amount of reduced glutathione was very small; and moreover, the continious time of decreasing was much short. Taking such the findings into consideration, the lowering of reduced glutathione content after the administration of nitroglycol may be assumed not because it is responsible for the inhibition of glucose-6-phosphate dehydrogenase, but because it is due to the direct oxidation of reduced glutathione by means of nitroglycol.

Nitroglycol behaves to erythrocytes oxidatively and as the result, the formation

of methemoglobin and heinz bodies is carried out. Consequently, it is presumed that nitroglycol also makes some effects on the carbohydrate metabolism. First, Aizawa and associaters[44] have reported that the content of blood sugar and lactic acid in their blood increased conspicuously in the time nitroglycol was administrated to dogs. The results in this experiment were almost in accord with those of Hasegawa et al. Namely, there was a marked increase in the content of blood sugar and lactic acid after the injection of nitroglycol. Blood sugar reached the maximum at 2 to 4 hours later and at that time, its concentration was 318 mg per 100 ml of the blood in the most remarkable case. On the other hand, lactic acid attained the maximum at 2 hours later and its concentration was 69.44 mg per 100 ml of the blood at the peak of the most conspicuous case.

As the substances effecting upon the carbohydrate metabolism, sodium salicylate,[45] 2-(2,6-dichlorophenylamino)-2-imidazoline,[46] hydrazine,[47] epinephrine,[48] amytal,[49] pentbarbital,[49] thiopental,[50] pilocarpine,[51] and ether etc.[52] have been known up to the present. However, their acting mechanism on the carbohydrate metabolism differs among them.[47][52][53] Therefore, in order to elucidate the metabolic impairment of carbohydrate by nitroglycol, it is necessary for the some enzymes concerning glycolisis and the content of epinephrine in medulla of suprarenal gland to develop further studies. But, because of the lack of data at present, a discussion on this respect here is omitted.

## SUMMARY

The author carried out some investigations on the intermediary metabolites and the biological effects of nitroglycol using rabbits. The results were as follows.

1) The content of free nitroglycol in the blood reached the maximum already at 1 hour after the injection. Thereafter, it began to decrease rapidly, became the trace amount at 6 hours later, and was completely eliminated from the blood at 24 hours later.

2) The content of nitrate increased slowly from immediately after the injection until 3 hours later, was kept approximately constant over 3 to 5 hours later, and then, decreased gradually.

3) The content of ethylene glycol in the blood did not vary within 1 hour after the injection. Thereafter, it increased promptly, reached the maximum at 2 to 3 hours later, and then, decreased immediately.

4) Methemoglobin was formed rapidly after the injection of nitroglycol. The content of methemoglobin in the blood reached the maximum at 1 to 3 hours later and at that time, 23 to 27 percent of methemoglobin in the case injected with 0.15 g of nitroglycol per kg of the body weight and 45 to 50 percent of methemoglobin in the case received 0.3 g as the same, respectively, were detected.

Thereafter, it began to decrease rapidly, fell to the trace amount at 8 hours later, and vanished completely from the blood at 24 hours later. On the other hand, in the successive administration daily, the content of methemoglobin at the peak increased from 1st to 2nd day, but after 3rd day, it decreased gradually. Furthermore, the forming rate of methemoglobin did not nearly change during this experimental period, but its diminishing rate decreased gradually. In especial, the lowering of the diminishing rate appeared conspicuously after the administration was stopped for 2 days.

5) The formation of methemoglobin after the administration of nitroglycol may be considered due to the direct oxidation by nitroglycol itself.

6) The activity of SGO-T increased remarkably after the administration of nitroglycol. On the contrary, there was no significant change in the activity of SGP-T.

7) The inhibition of catalase activity appeared rapidly after the injection of nitroglycol and at the least, continued for more than several hours. In addition, the activity of catalase at 24 hours later brought about the lowering with the frequency of injection.

8) After the administration of nitroglycol, the content of reduced glutathione slightly decreased and reached the minimum at 2 to 4 hours later. Thereafter, it increased gradually and at 24 hours later, was little higher than the pre-injection level.

9) There was a significant increase in the content of lactic acid in the blood. The content of lactic acid reached the maximum approximately at 2 hours later. Thereafter, it began to decrease and attained the maximum at 6 hours later. Again, it increased slowly, but did not reach the pre-injection level.

10) The content of blood sugar increased conspicuously after the injection of nitroglycol and reached the maximum about 2 to 4 hours later. Thereafter, it decresed gradually and reached to the pre-injection level or slightly below at 24 hours later.

A part of this studies was supported by the aid of research fund from the Ministry of Education, and presented at the 55 th Conference of the Medico-Legal Society of Japan, Tokyo, April, 4, 1971 and at the same 56 th Conference of Japan, Kyoto, April, 8, 1972. The author expresses him deep gratitude to prof. J. Furuno for his unfailing guidance through the course of this work and wishes to thank Nipponkayaku Co. for supplying of nitroglycol and all menbers of this laboratory for their assistance in the experimental work.

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