On the Separation and Identification of Drugs of Pylazolone Derivative by Thin-Layer Chromatography and Ultraviolet Absorption Spectrophotometry

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The accidents on account of the injection have nowadays occupied the great part of medical accidents,[1] of which the side effect of the injection itself, suppuration of the region injected, neuroparalysis as sequelae, mistake of an injection, and shock are involved. In general, the drugs to which lead the shock have been better known with anesthetic, antibiotic, pyrazolone derivatives, sulfa drugs, proteolytic enzymes, vaccine serum, BSP, and ACTH. In addition, the shock[2)3)] by using glucose and vitamin B_1 has been reported. Recently, we encountered a case that the cause of death was assumed to be the shock based upon pyrazolone derivatives and we have carried out the some experiments for the separation and identification of these derivatives.

CASE REPORT

History

This cadaver was an 82-year-old woman, who had not any special mentions for the family and past history. She felt a pain in the shoulder and after a week, consulted a doctor of K hospital in U city at 9.40 a.m. on the 14th of June, 1968. Under the diagnosis of periarthritis of right shoulder, 20 ml of an injection, "Narbron" containing pyrazolone derivatives was intravenously injected. The injection was inserted slowly. At 15 ml was injected, because she sneezed twice, the doctor stopped injecting for a while. After a little while, the injection was further continued inasmuch as she became calm and at the time 18 ml inserted she revealed the appearance that her throat was obstracted by sputum and began her arms moving. Therefore, the doctor was impossible to support a syringe in the normal manner and stopped the injection. As the dissputum seemed to be impossible for herself, sputum was artificially removed and her respiration resulted easy. But a few minutes later she suddenly became unconcious and her respiration ceased. Immediately, the artificial respiration was begun and after 5 minutes, the oxygen respiration was carried out through the anesthesia machine with closed circuit, whereas she died at 10.25 a.m., forty minutes later after the injection.

Anatomical Findings

Beginning of autopsy: At 9.45 a.m., on the 15th of June, 1968 (postmortem time was approximately 23.3 hours).

Gross Findings:

She was about 142.0 cm in length. The constitution was small and nutrition was bad. The lividity was in the back, showing usual dark red in color. On the face the congestion and edema were not observed. The conjunctivae were pale and petechial hemorrhage was not found on these. The marks of injection existed on the outside of the left upper arm and the right cubital fossa and the discoloration which is dark red in color and about a boad bean in size, was accompanied by near these. For organs, in the brain the remarkable change was not revealed. Subcapsule petechial hemorrhage of thymus was not seen and the parenchyma was completely converted into adipose tissue. For lymphatic tissue, the lymph nodes in the whole body, tonsils, and mesentery were moderately developed.

Heart: It was 230 g in weight, slightly larger than her fist in size and soft in hardness. The amount of epicardial fat was somewhat larger than usual. The size of coronary arteries were normal, showing no sclerotic change of the intima. There was the small amount of the dark red and fluid blood and not coagulated blood in the heart. The foams and the findings suspectable for air emborism were not observed. The thickness of heart muscle was approximately 1.0 cm in the left and 0.6 cm in the right. The valves showed no abnormalities. The width at the ostium aortae was approximately 7.0 cm and hypertrophic which was pale yellow in color and a millet seed in size was found in some parts of intima.

Lungs: The left lung was 440 g in weight and approximately 23.0 × 17.0 × 4.0 cm in size. The surface was red purple in color and rich in elasticity. The section through the lung was red brown in color and the flow amount of blood by pressure was moderate, but the flow amount of frothy fluid was somewhat larger than usual. The bronchial trees were filled with white frothy fluid. The right lung was 480 g in weight and approximately 25.0 × 15.0 × 4.0 cm in size. The color of the surface and section were about the same as the left. The middle lobe showed the appearance of emphysema.

Liver: It was 740 g in weight and 22.0 × 13.0 × 3.0 cm in size. It was atrophic and brittle. The surface was smoth and red brown in color. Section through the liver revealed a brownish parenchyma with somewhat great amounts of blood. Each small lobule was well distinguished.

Kidneys: They were 130 g and 110 g in weight and 10.0 × 6.0 × 2.5 cm and 9.5 × 5.5 × 3.0 cm in size, respectively in the left and right. The capsules stripped with ease leaving a smoth, violet surface. The sections were moist with the dark red medulla having radiating red streaks and being demarked with pale, thin corteces. The flow amount of blood by pressure was somewhat large. The kidney pelvis was not remarkable.

Adrenals : They were normal.

Spleen : It was 40 g in weight, $8.5 \times 6.0 \times 1.0$ cm in size. The surface was greyish violet with the capsule wrinkled. On section, the parenchyma was red violet. The trabecule and follicle was well distinguished.

Pancreas : It was 16.0 cm in length and remarkable change was not found.

Stomach, intestine, bladder, and genital organs was not remarkable.

Nasal cavity and upper part of pharynx : The hyperplasia of mucosa and edema was not recognized.

Microscopic Findings

Heart : The miocardium fibers were as a rule atrophic and the hypertrophia was observed in several parts. Around the nucleus, the appearance of lipofuscin was remarkable. The fragmentation of miocardium was seen everywhere. Most parts of the wall of blood vessel were relatively thickened and the interstitial connection tissue revealed the appearance of edema. The change due to inflammation, fatty change, vacuoler change, and strongly stained parts with eosin were not observed, respectively.

Lungs : The lung appeared edema both in the left and right. The small blood vessel was enlarged and filled with erythrocytes. Around the blood bessel, the infiltration of lymphocytes were seen in several parts. A part revealed the appearance of emphysema. There were not any findings due to the inflammation.

Liver : The remarkable change was not recognized concerning the constitution of hepatic lobule. The degeneration was seen in the parts near cell center in the hepatic cells were light and smallish, and the appearance of lipofuscin was recognized, but the necrosis was not found. In the peripheral parts of lobules, the fatty change was observed. There were not any noticeable signs about the Kupffer cells and Glison's capsule.

Kidneys : The arteriosclerotic degeneration was found. The change of glomerulus and renal tubles was scarecely seen. The wall of small blood vessel was enlarged. The intima was irregularly thickened and the stenosis of vessel was partialy seen. Mostparts of the venous capillaries were enlarged. The chang based upon inflammation was not observed.

Adrenals : The cortex was atrophic. The zona glomerulosa was thin. The zona fasciculosa was moderate. The zona reticularis was widespread. The blood vessel of medulla was enlarged and the inflammatory reaction was not recognized.

EXPERIMENT

Materials and Methods

1. Materials

Standard materials : Sulpyrin, aminopyrine, caffeine, sodium salicylate, and sodium bromide.

Materials for testing: An injection, "Narbron" (manufactured by Hitachi chemical company and in 20 ml, 0.4 g of a mixture of sulpyrin and aminopyrine, 0.1g of sodium salicylate-caffeine, 0.4 g of sodium salicylate, and 0.3 g of sodium bromide were contained) and heart blood (38 ml).

2. Preparation of plates

Thirty g of silica gel G was placed in a mortar, to this was added 60 ml of pure water during stirring slowly, and a mixture was well slurried until uniform. The slurry was poured onto the glass plate and spreaded uniformly by a rod of glass equipped with 250 mμ thickness of cellophane tape to its both edges. After left at room temperature for several hours the coated plates were activated in an oven at 110 $\pm$ 10°C for an hour and then transferred to the stage containing the desiccant.

3. Developing procedure

The solution of materials were spotted onto at point of 2 cm apart from one end of the plate. The development was carried out by ascending technique in the chamber which was previously saturated with the solvent used.

4. Detecting reagents

Dragendorff's reagent[4]: Solution A was made by addition of 40 ml of pure water to the solution which 0.85 g of bismuth nitrite was dissolved in 10 ml of glacial acetic acid. Solution B was prepared by dissolving 8 g of potassium iodide into 20 ml of pure water. Before using the equal volumes of solutions A and B and glacial acetic acid were mixed.

Potassium permanganate reagent: Two % aqueous solution of potassium permanganate.

Ehrlich's reagent: Two % solution of p-dimethyl aminobenzaldehyde in 6 % hydrochloric acid.

Diazonizing reagent[5]: Two N hydrochloric acid, 2 % aqueous solution of sodium nitrite, and 0.02 % alcoholic solution of N-ethyl-α-naphthylamine each was prepared. Diazonizing on the plate was carried out by spraying hydrochloric acid followed by sodium nitrite and coupling with N-ethyl-α-naphthylamine.

Iodine and alcoholic hydrochloric acid reagent: The plate was first exposured to iodine vapour and followed by spraying a mixture of 7 ml of 25 % hydrochloric acid and 1 ml of 96 % ethyl alcohol.

Uranine reagent[6]: A mixture of 1 ml of 1 % fluorescine sodium solution, 5 ml of glacial acetic acid, and 5 ml of 3 % hydrogen peroxide solution was prepared immediately before using. For detecting, a mixture solution and 2 N sodium hydroxide were sprayed on the plate in that order. Thus treated plate was heated 100° C for 30 minutes in an oven.

Schiff's reagent: One-tenth g of fuchsin was dissolved in 60 ml of boiling water. After the solution being cooled at room temperature, to this were added 10 ml of 1 % anhydrous sodium bisulfite solution and 1 ml of concentrated hydrochloric

acid. The solution was then made to 100 ml of volume with pure water. After stirring well, the solution was allowed to stand at room temperature and supplied for use as needed.

Bromthymolblue reagent : Forty mg of bromthymolblue was dissolved in 40 ml of ethyl alcohol. To this was added the diluted solution of sodium hydroxide until the solution turned blue.

Mercurous nitrate reagent: One % aqueous solution of mercurous nitrate.

Chlorine and potassium iodide-starch reagent[7] : After exposured to chlorine vapour, the plate was freed from an excess of chlorine and followed by spraying the solution containing each 1 g of starch and potassium iodide in 100 ml of pure water.

Results and Discussion

1. Studies on detecting reagents

After each 5 μl of the aqueous solutions (ranged from 0.05–1.0 μg/μl) of constituents of an injection, "Narbron", such as sulpyrin, aminopyrine, sodium salicylate, caffeine, and sodium bromide was spotted onto the activated plate, the different reagents mentioned before were on the spotted plate and the studies were carried out on the behavior reagents, the color of spots and limits of detection, respectively. These results are shown in Tables 1–5.

Table 1. Aminopyrine

	5μg	2.5μg	1μg	0.5μg	0.25μg
1	+	+	+	+	±
2	+	+	+	+	±
3	+	±	−	−	−
4	−	−	−	−	−
5	+	+	+	+	±
6	+	+	+	±	−
7	+	+	±	−	−
8	−	−	−	−	−
9	+	±	−	−	−

Reagent 1: Dragendorff's,
Reagent 2: Potassium permanganate,
Reagent 3: Palladium chloride,
Reagent 4: Diazonizing,
Reagent 5: Iodine Vapour,
Reagent 6: Iodine and alcoholic hydrochloric acid,
Reagent 7: Chlorine and potassium iodide-starch,
Reagent 8: Ehrlich's,
Reagent 9: Ferric chloride.

Table 2. Sulpyrin

	5μg	2μg	1μg	0.5μg	0.25μg
1	−	−	−	−	−
2	+	+	±	−	−
3	+	+	±	−	−
4	−	−	−	−	−
5	+	+	+	+	±
6	+	+	+	±	−
7	+	±	−	−	−
8	+	+	+	±	−
9	+	+	±	−	−

Reagents: The same in the Table 1.

Table 3. Salicylate

	5μg	2.5μg	1μg	0.5μg	0.25μg
1	+	+	+	±	−
2	+	+	+	+	±
3	+	+	+	+	+
4	+	±	−	−	−
5	±	−	−	−	−

Reagent 1: Potassium permanganate, Reagent 2: Ferric chloride, Reagent 3: Iodine vapour, Reagent 4: Bromthymolblue, Reagent 5: Mercurous nitrate.

Table 4. Caffeine

	5μg	2.5μg	1μg	0.5μg	0.25μg
1	+	+	+	+	+
2	±	−	−	−	−
3	−	−	−	−	−

Reagent 1: Iodine vapour, Reagent 2: Iodine and alcoholic hydrochloric acid, Reagent 3: Ferric chloride.

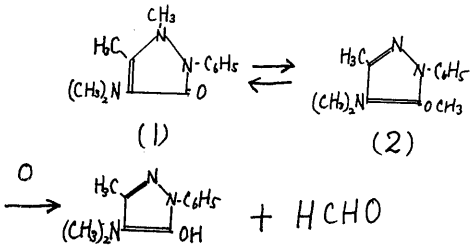
Table 5. Bromide

	5μg	2.5μg	1μg	0.5μg	0.25μg
1	−	−	−	−	−
2	+	±	−	−	−
3	−	−	−	−	−
4	−	−	−	−	−
5	−	−	−	−	−

Reaegnt 1: Schiff's, Reagent 2: Uranine, Reagent 3: Iodine vapour, Reagent 4: Ferric chloride, Reagent 5: Potassium permanganate.

Potassium permanganate, iodine vapour, and iodine-alcoholic hydrochloric acid were sensitive to both aminopyrine and sulpyrin ; Dragendorff's to aminopyrine ; Ehrlich's to sulpyrin ; iodine vapour, ferric chloride, potassium permanganate to sodium salicylate ; iodine vapour to caffeine ; uranine to sodium bromide. Of these reagents, iodine vapour reacted with all the other compounds except sodium bromide to color and as a rule, was superior to another reagent in point of the limits of detection, whereas, the color of spot was not any differences among constituents and very rapidly disappeared. On the other hand, ferric chloride reagent was less sensitive than iodine vapour, but it gave different color spots, for example, aminopyrine purple, sulpyrin pink, sodium salicylate violet, caffeine red earth, and sodium bromide orange, and the outline of the individual spots were remarkable distinct. Consequently, ferric chloride reagent appeared to be most suitable reagent for the simultaneous analysis of all constituents in an injection.

The color reaction mechanism of all constituents with ferric chloride may be assumed as followed. In case aminopyrine and sulpyrin, they first are oxidized by ferric chloride, the resulting products combine with ferric ion, and then the color reaction substances seem to be formed. Namely, as shown in a scheme below, aminopyrine is considered to consist of both isomers[8] of (1) and (2) due to the action of dimethylamino group. Of both isomers, methoxy group of isomer of (2) is oxidized by ferric chloride, converting into hydroxyl group and formaldehyde. On the other hand, the resulting oxypyrazole combines with ferric ion to form the color reaction product.



The color reaction mechanism of sodium salicylate and ferric chloride exists in the formation of inner complex salt. Hence, the other two isomers, m- and p-oxybenzoic acid do not color with ferric chloride. On the contrary, in case sodium bromide, it is converted into bromide due to the action of ferric ion and bromide seems to represent its own color.

$$2\,Br^- + 2\,Fe^{3+} \longrightarrow Br_2 + 2\,Fe^{2+}$$

2. Studies on developing solvents

For the purpose of selecting the solvents suitable for the simultaneous analysis of five constituents in an injection, "Narbron", the authors have studied on the five solvents, such as acetone-n-buthanol-concentrated ammonia water-water (65 :

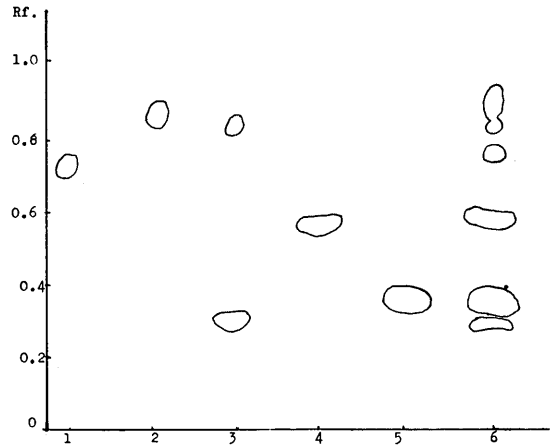
Table 6. Rf values of five consituents

	Rf Values				
	Solv. 1	Solv. 2	Solv. 3	Solv. 4	Solv. 5
Aminopyrine	0.88	0.77	0.49	0.82	0.46
Sulpyrin	0.49	0.27	0	0.06	0
Sulpyrin decomp.	0.85	0.73	0.31	0.80	0.30
Salicylate	0.70	0.40	0	0.04	0.03
Caffeine	0.75	0.61	0.30	0.78	0.31
Bromide	0.40	0.21	0	0	0

Solvent 1: Me_2CO-n-BuOH-conc. NH_4OH-H_2O (65 : 20 : 10 : 5 v/v %)
Solvent 2: Upper layer of n-BuOH-H_2O-conc. NH_4OH (100 : 66 : 33 v/v %)
Solvent 3: Me_2CO-Benzol-conc. NH_4OH (40 : 60 : 1 v/v %)
Solvent 4: $CHCl_3$-EtOH-conc. NH_4OH (80 : 20 : 2 v/v %)
Solvent 5: Me_2CO-$CHCl_3$ (60 : 40 v/v %)

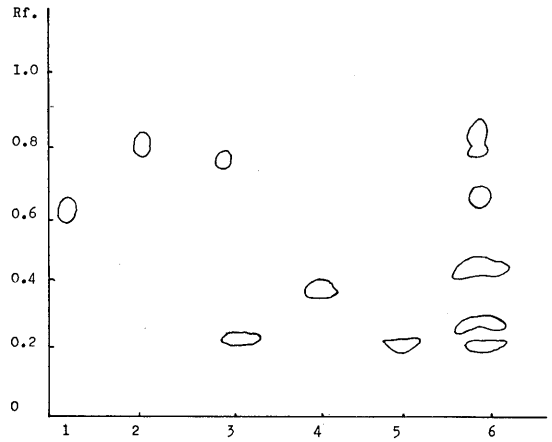
20 : 10 : 5 v/v %), upper layer of n-buthanol-water-concentrated ammonia water (100 : 66 : 33 v/v %)[9], acetone-benzol-concentrated ammonia water (40 : 60 : 1 v/v %), chloroform-ethanol-concentrated ammonia water (80 : 20 : 2 v/v %), and acetone-chloroform (60 : 40 v/v %). Rf values in these solvents are shown in Table. 6. Chromatograms of aminopyrine, sulpyrin, sodium salicylate, caffeine, and sodium bromide with a mixture of acetone-n-buthanol-concentrated ammonia water-water (65 : 20 : 10 : 5 v/v %) are illustrated in Fig. 1 and chromatograms of these in upper layer of a mixture of n-buthanol-water-concentrated ammonia water (100 : 66 : 33 v/v %) in Fig. 2, respectively.

Fig. 1. Thin-layer chromatograms of five constituents



Solvent: Me_2CO-n-BuOH-conc. NH_4OH-H_2O (65 : 20 : 10 : 5 v/v %)
Detecting reagent: Ferric chloride (9 %)
Constituents: 1) Caffeine, 2) Aminopyrine, 3) Sulpyrin,
4) Salicylate, 5) Bromide, 6) Mixture

Fig. 2. Thin-layer chromatograms of five constituents



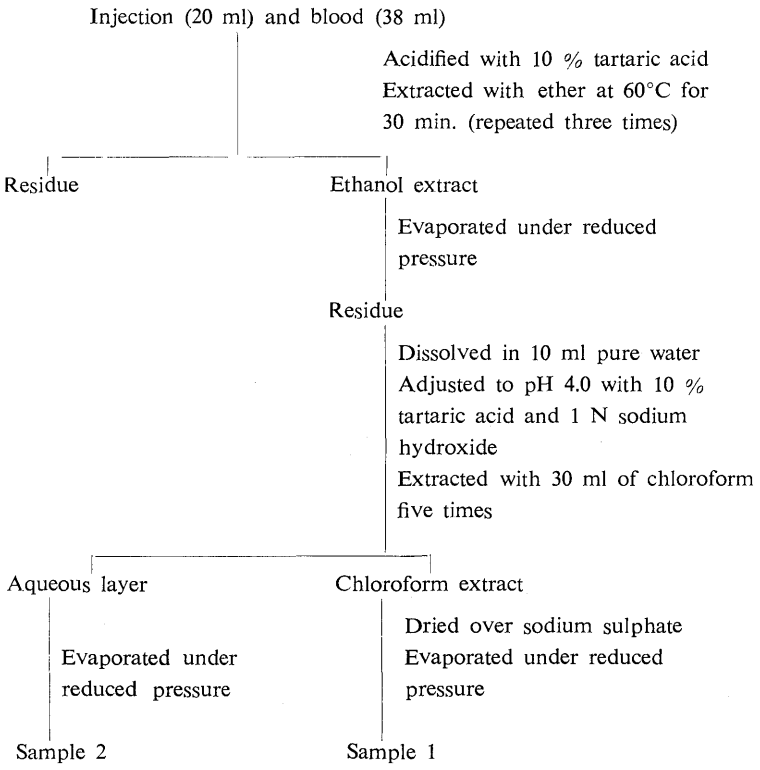
Solvent: Upper layer of n-BuOH-H_2O-conc. NH_4OH (100 : 66 : 33 v/v %)
Detecting reagent: Ferric chloride (9 %)
Constituents: 1) Caffeine, 2) Aminopyrine, 3) Sulpyrin, 4) Salicylate, 5) Bromide, 6) Mixture

In general, the higher the polarity of solvent is, the more the chromatogram ascended. The satisfactory separations were obtained by useing upper layer of n-buthanol-water-concentrated ammonia water (100 : 66 : 33 v/v %) and acetone-n-buthanol-concentrated ammonia water-water (65 : 20 : 10 : 5 v/v %) and the spots of these constituents were sufficiently separated to allowed easy identification. But, when the mixtures of acetone-benzol-concentrated ammonia water (40 : 60 : 1 v/v %), chloroform-ethanol-concentrated ammonia water (80 : 20 : 2 v/v %), and acetone-chloroform (60 : 40 v/v %) were used as solvents, the satisfactory separations of these would not be expected and the spots of sulpyrin, sodium salicylate, and sodium bromide remained at or near the position of spotting. The phenomena of the diffused spot and tailing were not observed throughout all the solvents.

3. Examination of medico-legal object

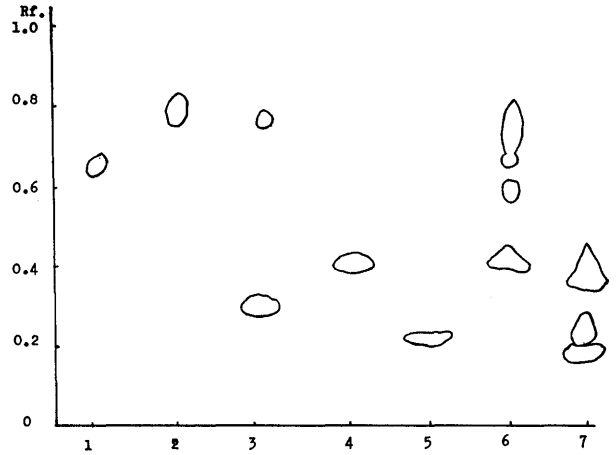
Stas-Otto's technique has been well known as a method for extracting drugs and poisons since olden times and nowaday applied in the field of forensic chemistry. But, because most of constituents in "Narbron" injection (aminopyrine, sulpyrin, sodium salicylate, caffeine, and sodium bromide) are slightly soluble in ether, it was considered that the direct application of Stas-Otto's technique to these constituents might be inadequate, especially in the recovery. Watanabe[10] has recently studied on the recovery of a number of drugs by using individual solvents. According to his work, the recovery of these constituents in general are high when chloroform and ethylene dichloride are used, but low with ether.

Fg. 3. The extraction of aminopyrine, sulpyrin, caffeine, salicylate, and bromide in an injection, "Narbron" and blood



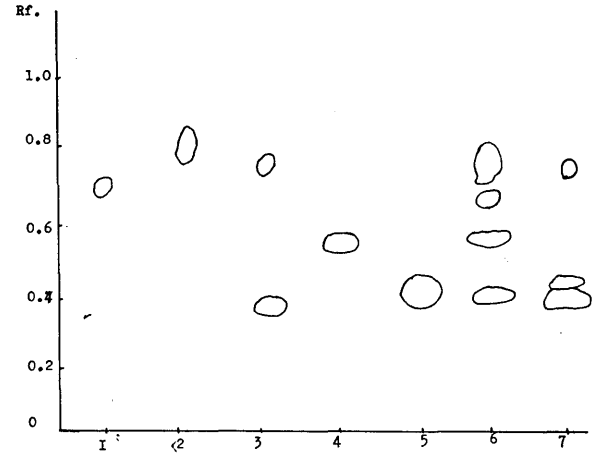
Therefore, as shown in Fig. 3, we modified a part of Stas-Otto's extracting scheme and according to this, attempted extracting five constituents from each 20 ml of an injection and 38 ml of heart blood. In the case an injection, the extract from chloroform phase was yellow brown and viscid matter and that from aqueous phase was pale yellw crystals. In case blood, the extract from chloroform phase was pale yellow matter and that from aqueous phase was the same color matter (a part like crystals). The extracts from aqueous phase were both positive for Beilstein's test. Subsequently, the extract from chloroform phase (sample 1) and that from aqueous phase (sample 2) of an injection or blood and standard samples (aqueous solution) spotted onto the same plate and the spots were developed by one dimensional and ascending technique with both the mixtures of upper layer of n-buthanol-water-concentrated ammonia water (100 : 66 : 33 v/v %) and acetone-n-buthanol-concentrated ammonia water-water (65 : 20 : 10 : 5 v/v %). After development, the plate was allowed to stand at room temperature in order to remove an excess of solvent and followed by staining with ferric chloride reagent.

Fig. 4. Thin-layer chromatograms of constituents extracted from an injection, "Narbron"



Solvent: Upper layer of n-BuOH-H_2O-conc. NH_4OH (100 : 66 : 33 v/v %)
Detecting reagent: Ferric chloride (9 %)
Control: 1) Caffeine, 2) Aminopyrine, 3) Sulpyrin, 4) Salicylate, 5) Bromide
Injection extract: 6) Sample 1 (transferred into chloroform layer),
7) Sample 2 (remained in aqueous layer)

Fig. 5. Thin-layer chromatograms of constituents extracted from blood



Solvent: Me_2CO-n-BuOH-conc. NH_4OH-H_2O (65 : 20 : 10 : 5 v/v %)
Detecting reagent: Ferric chloride (9 %)
Control: 1) Caffeine, 2) Aminopyrine, 3) Sulpyrin, 4) Salicylate, 5) Bromide
Blood extract: 6) Sample 1 (transferred into chloroform layer)
7) Sample 2 (remained in aqueous layer)

The chromatograms are illustrated in Figs. 4–5. From sample (1) of an injection, aminopyrine, decomposed matter of sulpyrin (seems to be 4-methy-aminoantipyrine or methylene-bis-methyl-aminoantipyrine), caffeine, and salicylate and from sample

(2), sulpyrin, salicylate, and bromide were identified, respectively. From sample (1) of blood, aminopyrine, decomposed matter of sulpyrin, salicylate, bromide and caffeine and from sample (2), sulpyrin, decomposed matter and bromide were identified.

For the sake of separating and purifying the extracts into individual constituents, each sample was repeatedly spotted onto the plate as a streak and again subjected to the chromatography using upper layer of a mixture of n-buthanol-water-concentrated ammonia water (100 : 66 : 33 v/v %) as solvent. After drying in air, ferric chloride reagent was poured onto one of the thin film and the developed positions of constituents were distinctly marked according to the chromatograms. The thin film was scraped from the glass plate and received on a glass filter and the constituents each was eluted with warm water. The crystals of individual constituents obtained from an injection were further recrystallized from water and ethyl alcohol, but those from blood could not be subjected to the further purification because of the small quantity of crystals.

Subsequently, owing to raising the reliability, the measurement through ultraviolet spectrophotometry[11] was performed in addition to the thin-layer chromatography. The materials which were chromatographically identified as aminopyrine, sulpyrin, caffeine, and salicylate each was dissolved in pure water and the spectra of these solutions were measured using pure water as a blank. The material recognized as bromide was dissolved in 2 ml of pure water and to this were added 2 ml of 6 N sulphuric acid and "knife-point" of potassium permanganate and 5 ml of n-cyclohexane. The mixture was shaked vigorously for 20–25 seconds and centrifuged. The upper layer were measured against the blank which run through the same procedure using 2 ml of pure water free from bromide. These results are shown in Figs. 6–10.

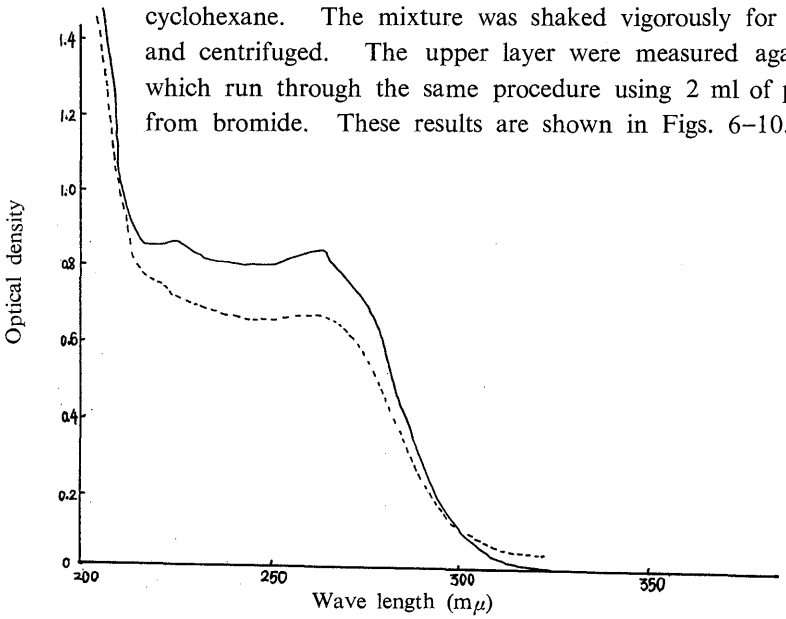


Fig. 6. Absorption spectra of aminopyrine extracted from an injection, "Narbron" and blood in aqueous solution. —— Injection, ····· Blood.

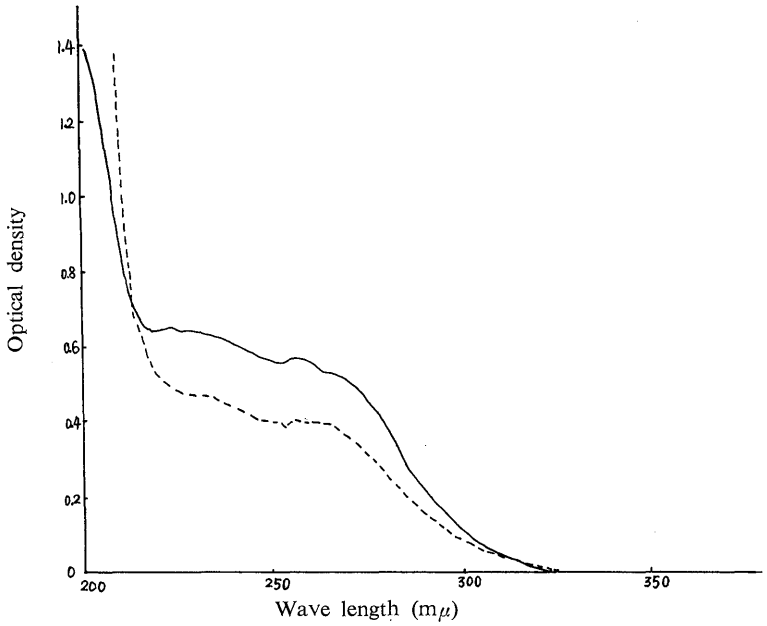


Fig. 7. Absorption spectra of sulpyrin extracted from an injection, "Narbron" and blood in aqueous solution. ——Injection, ……Blood.

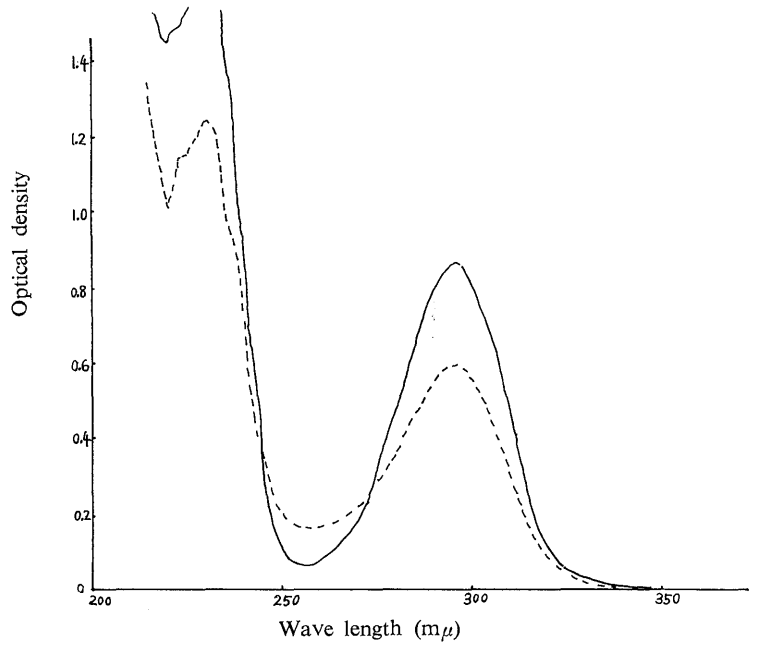


Fig. 8. Absorption spectra of salicylate extracted from an injection, "Narbron" and blood in aqueous solution. ——Injection, ……Blood.

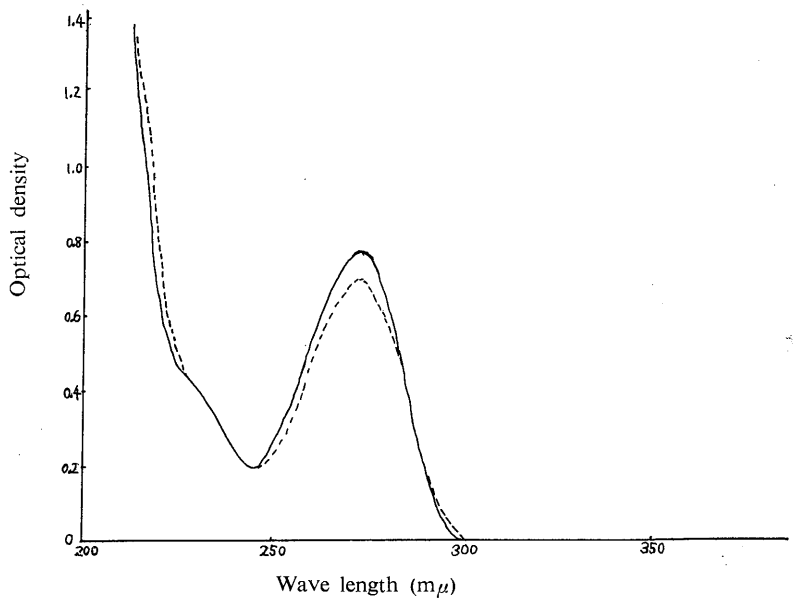


Fig. 9. Absorption spectra of caffeine extracted from an injection, "Narbron" and blood in aqueous solution. ——Injection, ……Blood.

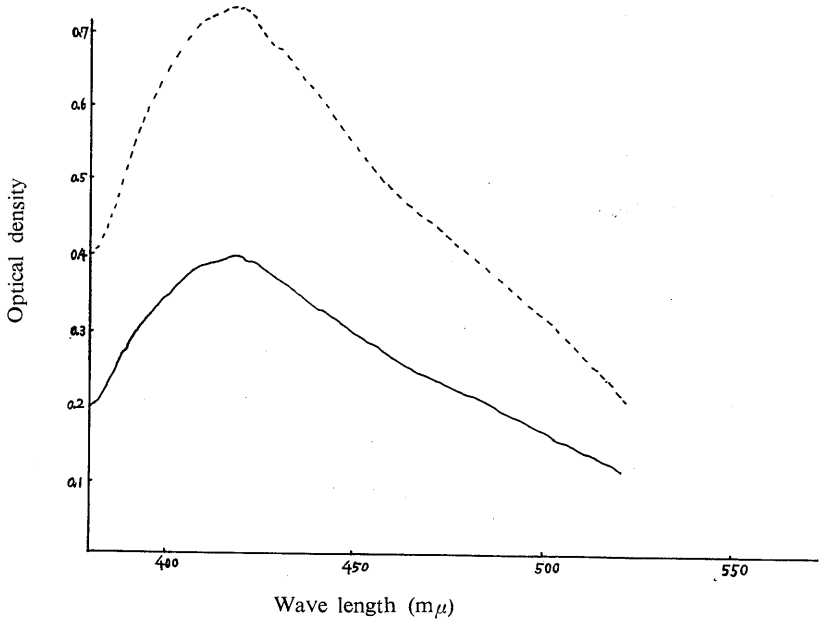


Fig. 10. Absorption spectra of bromide extracted from an injection, "Narbron" and blood in n-cyclohexane. ——Injection, ……Blood.

The spector of each constituent was as follows. Aminopyrine (injection λmax. 263 mμ : blood λmax. 263 mμ), sulpyrin (nothing absorption at the specified wave length), salicylate (injection λmax. 295 mμ and λmin. 256 mμ : blood λmax. 295.5 mμ and λmin. 256 mμ), caffeine (injection λmax. 273 mμ and λmin. 245 mμ : blood λmax. 273 mμ and λmin. 245 mμ), bromide (injection λmax. 420 mμ : blood λmax. 419 mμ). The spectra patterns of five constituents closely agreed with these of standard materials.

COMMENT

Since the before century, the mechanism leading to the shock has been studied in all respects. Reilly and Freeman has suggested the sympathicotonia. Wiggers, Blalock, and Hankins have emphasized the theory of the lacking of local body fluid. Selye has described the stress theory, chiefly based upon the balance of adreno-corticotrophic hormone and corticoid, "general adaptation syndrome". Thus, although the pathological physiology of the shock is not completely proved, the main symptom has been considered to be the striking change of the circulation state on account of the rapid lowering of arterial blood pressure. Namely, the decreasing of blood volume and the lowering of blood pressure which flow back from vena cava to right auricle, play the part of the deciding factors of symptom and bring the disturbance of peripheral circulation.

The main anatomical findings of this cadaver are as follows. There were edema and emphysema in lungs and arteriosclerosis in kidneys. The findings of air embolism and like the status thymicolyphaticus were not seen. The blood in heart was fluid and dark red. The inflammatory reaction was not found at all in organs. The capillary vessels, especially in the side of venous capillaries, were enlarged and filled with blood. The hepatic cells were in the beginning of degeneration and the findings of the lower nephron nephrosis were not observed. The cause of death might be assumed to be a primary shock on the basis of anatomical findings mentioned before.

Because pylazolone derivatives possess the relatively high antigenicity, it has been already known that they frequently acts on man to be allergic and moreover, that they are likely to appear the antigenicity only when combining with protein as haptene.[12)13)14)] Whereas, Ogata[15)] has suggested that the evidence is insufficient in order that the shock induced by using drugs containing penicillin may be responsible for antigen-antibody reaction. Goetz[16)] and Kalz[17)] have had some doubts confirming such a anaphylactic shock, and Iwata[18)19)] has interpreted it merely as Reilly phenomene.

Taking the various things mentioned above into consideration, to touch on the shock by using pylazolone derivatives itself, here, is considered inadequate. In case this cadaver, the skin tests for allergy were not carried out, the past history for

pyrazolone derivative was utterly unknown, and further, a period between active sensitization and death comes into question.

According to the report[2)3)] for the autopsies investigated by the medicolegal society of Japan, the fatal shock is of greatest under the administration of anesthesia and antibiotic, followed by the dosage of pyrazolone derivatives. As the causative factor[2)3)20)21)22)] in such a case, besides the disposition of allergy, status thymicolimphaticus and heart disease are frequently observed in the autopsy and the findings of hepatosis and hypocorticalism, which are worth for note, especially in the oral administration,[3)] are not a few.

CONCLUSION

The authors have reported for the autopsy of a cadaver who had developed the serious symptom during the injection of drug containing pylazolone derivatives and forty minutes later died, and descrived for the separation and identification of constituents in an injection by means of thin-layer chromatography and ultraviolet absorption spectrophotometry.

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