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Physico-Chemical Properties and Local Toxic Effects of Injectables

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OSHIDA, S., DEGAWA, K., TAKAHASHI, Y. and AKAISHI, S. *Physico-Chemical Properties and Local Toxic Effects of Injectables.* Tohoku J. exp. Med., 1979, **127** (4), 301-316 —— In Japan, many cases of muscle contracture as a sequela of injections have been reported. We studied the physico-chemical properties and muscle-damaging potential of many injectables which are commonly used in hospitals. Contrary to our expectations, the pH of the injectables was found to range widely from 1.4 to 12.8, and the osmotic ratio from 0.2 to 36. It was also found that their hemolytic potential was closely related to the severity of the muscle lesions in animal experiments and that there were many injectables with strong muscle-damaging potentials. Therefore, doctors should be informed of the physico-chemical properties and tissue-damaging potential of each injectable; pharmaceutical companies should exert all possible efforts to improve injectables; and doctors should keep the administration of intramuscular injections to a minimum and use them only in cases of actual need. ——— injectables; pH; osmotic ratio; hemolysis; muscle lesion

The injection has been one of the most popular medical techniques since the early stage of modern medical history. It is said that intravenous injection was invented by Major in 1662, subcutaneous injection by Wood in 1853, and intramuscular injection by Luton in 1882 (Oshida 1973).

Since drugs, especially injectables, play one of the most important roles in medical treatment, medical disputes between doctor and patient are frequently connected with injection (Akaishi and Oshida 1972).

According to a survey by the Legistration Committee of the Japan Medical Association (1971), there were 1,640 cases of medical disputes from 1962 to 1970, in which accidents associated with injection were most frequent (539 cases, 33%) and these accidents included 206 cases of peripheral nerve injuries, 136 cases of shock, 99 cases of infection, and 98 cases of others. Further, Oshida et al. (1975) collected 953 cases of accidents due to injection from the literatures concerned, mainly from the orthopedic ones, which included 389 cases of radial nerve palsy, 61 cases of sciatic nerve palsy, and 31 cases of peroneal nerve palsy. It is evident that these cases are nothing but 'the tip of the iceberg'. For the prevention of such absurd accidents due to injection, Akaishi and Oshida (1972)

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pointed out errors in injection sites commonly indicated in nursing textbooks and some medical books published in Japan and in Western countries.

Among the cases of nerve paralysis due to injection, there are both extremes, that is, easily curable cases and incurable ones in spite of the fact that in either case the injection was administered forcibly though the patient claimed a severe pain at the moment of needle puncture. We had doubt in regard to the reason of this difference, and assumed that there might be a considerable difference in the local action of the injectables used. Therefore, we attemped to analyze injectables, but we could not afford to buy many injectables. In consequence, in 1972 we asked the directors of 10 national or public hospitals in our district to give us injectables commonly used in each hospital, and received various kinds of injectables. Then, we began to analyze the physico-chemical and biological properties of injectables.

At that time, we were aware of the muscle contracture, which was said to be congenital or due to injection. After a while, a mass occurrence of the quadriceps femoris muscle contracture in children was brought to light in Yamanashi Prefecture in 1974 (Ohta 1974). Since then, many cases of the quadriceps femoris muscle contracture in children have been reported in Japan. According to the Medical Affairs Bureau of the Ministry of Health and Welfare, Japan (1976), there were 3,669 patients with the quadriceps femoris muscle contracture as of the end of December 1976. In addition, 8,631 cases with dimple or local induration in the femoral region but without any clinical symptom of muscle contracture have been reported.

Among the cases of contracture mentioned above, there are cases where injections of antipyretics and analgesics and/or antibiotics were administered only several times within several months or a few years after birth. These cases seem to suggest that some injectables have a fairly severe incompatibility with human tissue (Akaishi et al. 1974).

In this way, our study of injectables was linked directly to the problem of the muscle contracture due to injection.

Materials and Methods

Injectables commonly used in hospitals were examined for pH, osmotic pressure, hemolysis, toxic effect on cultured cells, and effect on the muscle tissue.

pH

The pH of the injectables was measured by the glass electrode method using Hitachi D5 pH meter and Beckman LABOMATE.

Osmotic pressure

The osmotic pressure of injectables was estimated by the freezing point method using AMCO 2077 Osmette and Advance L-type osmometer, and the osmotic ratio of each injectable was determined with the osmotic pressure of normal saline as 1.

Hemolysis

Macroscopic observation. One ml of each injectable incubated beforehand at 37°C for about

2 min, in company with the controls of normal saline and distilled water, was mixed with 0.1 ml of fresh human whole heparinized blood. The mixture was then incubated for 30 min at 37°C, centrifuged for 5 min usually at 3,000 rpm, and observed grossly for the presence or absence of hemolysis. The criteria for evaluation were as follows: (−), no hemolysis; (+), slight hemolysis; (+)′, slight hemolysis with discoloration; (卄), hemolysis; (卄)′ hemolysis with discoloration.

Quantitative hemolysis test with ^{51}Cr-labeled RBC. Human RBC were labeled with ^{51}Cr, then mixed with each injectable, and incubated at 37°C for 30 min. After the total radioactivity was counted, the mixture was centrifuged at 3,000 rpm for 5 min, and the radioactivities of the supernatant and precipitate were counted. Hemolytic ratio was determined as follows:

$$\text{Hemolytic ratio} = \frac{\text{count of supernatant}}{\text{total count}} \times 100$$

Toxic effects on cultured cells

Hela S3 cells were cultured in plastic Petri dishes with a diameter of 5 cm (Toyoshima Seisakusho) for 2–3 days. The monolayers of cells thus cultured were used for the present study. The culture medium employed was Eagle MEM supplemented with 10% bovine serum, and incubation was carried out in a carbon dioxide incubator.

The monolayer of cells sticking tightly to the bottom of each Petri dish was washed once with phosphate buffered saline (PBS) and cleared sufficiently of moisture. Using a syringe with a 23G×1 needle, a drop (about 0.01 ml) of injectables was dripped onto the center of the monolayer cells in the Petri dish. The mixture was immediately incubated in a CO_2 incubator at 37°C for 30 min. To each Petri dish then washed three times with PBS, a fresh culture medium was added, and incubation was continued until the following day. Then, the culture medium was removed, and after washing once with PBS, the cells were stained with 0.01% neutral red.

In evaluation of the results, not only the extent of cellular detachment after staining with neutral red and the status of cellular staining were determined macroscopically, but also any possible cytotoxic effect (CTE) was observed under a microscope. On the basis of findings thus obtained, CTE of injectables on cultured cells was evaluated in the following four grades:

(−): No cellular detachment is observed, and individual cells undergo no morphological change.

(+): Mild cellular detachment is observed, but the cells surviving in the drug-exposed area are stained with neutral red without any CTE being noted.

(卄): Cellular detachment is observed to a varying extent and a marked CTE is noted in the drug-exposed area. But, the CTE may be sometimes partial, and recovery is made after overnight incubation.

(卅): Cellular detachment is usually observed, though its extent varies with drugs. A marked CTE is noted in the drug-exposed area and no recovery is made after overnight incubation.

Local action to the muscle tissue

Male albino rabbits, weighing about 2 to 3.5 kg, were used, because the muscles are white and convenient for both macroscopic and microscopic observations. As a general rule, a single intramuscular injection of 0.5 ml of each injectable with a needle of 26G×1/2 was given into the vastus lateralis or sacrospinalis muscle. On the 3rd or 7th day of injection, the rabbits were exsanguinated to death, and the injected site was opened and the muscle was extirpated. Then, the muscle was cut transaxially every 3 to 5 mm in thickness, and congestion, bleeding, swelling, discoloration and necrotic changes were macroscopically examined. Then, these specimens were fixed and stained with hematoxylin eosin, Mallory's and/or Azan's staining to examine microscopically if there were any findings of bleeding, edema, cellular infiltration, degeneration, necrosis, or fibrosis.

From these findings, macro- and microscopical, the muscle lesion was classified into the following five categories: (−), no change observed; (+), very slight changes observed; (±), slight changes observed; (±), moderate changes observed; (±), marked changes observed.

As the control for (−), the same volume of normal saline was used, and for (±), 6% acetic acid solution was used.

In addition, for the long-term observation, the rabbits injected were examined a month after a single injection, or 3, 6 and 12 months after injections given daily for 5 consecutive days, or 3 times weekly for 4 consecutive weeks.

Results

pH of injectables

The pH of 304 kinds of injectables was found to be broadly distributed as shown in Fig. 1. The lowest pH was 1.4 of a tetracycline hydrochloride, and the highest was 12.8 of a sterile phenytoin sodium. The mode was 6.0 to 6.9 (105 injectables, 35%), followed by 5.0 to 5.9 (56 injectables, 18%) and 7.0 to 7.9 (53 injectables, 17%).

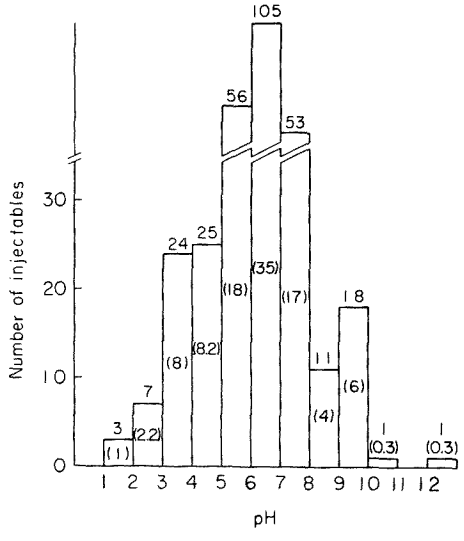

Fig. 1. The pH of 304 injectables. Percentages are given in the parentheses.

Dividing the injectables into subcutaneous, intramuscular and intravenous ones, no significant difference in pH was observed among them.

Osmotic ratio of injectables

The osmotic ratio of 300 kinds of injectables broadly ranged from 0.2 to about 36 of oxytetracycline preparations as shown in Fig. 2. No significant difference was observed among the three groups of injectables as mentioned above.

Further, according to our results of hemolysis test using NaCl solutions of serial concentrations, NaCl solutions of osmotic ratio from 0.5 to 7–8 gave no hemolysis.

Hemolytic potential of injectables

The results of macroscopic hemolysis test of 335 kinds of injectables are shown in Fig. 3, in which the intramuscular injectables are represented with oblique lines. As a whole, hemolysis (—) was 52%, (+) 12%, (+) with discoloration 1%, (±) 15%, and (±) with discoloration 20%. It is to be noted that many of injectables for intramuscular use showed strong hemolysis. The results of

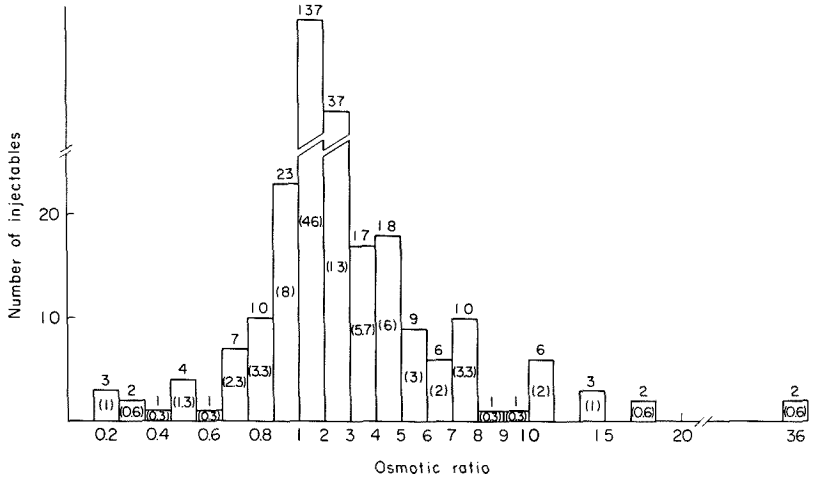

Fig. 2. The osmotic ratio of 300 injectables. Percentages are given in the parentheses.

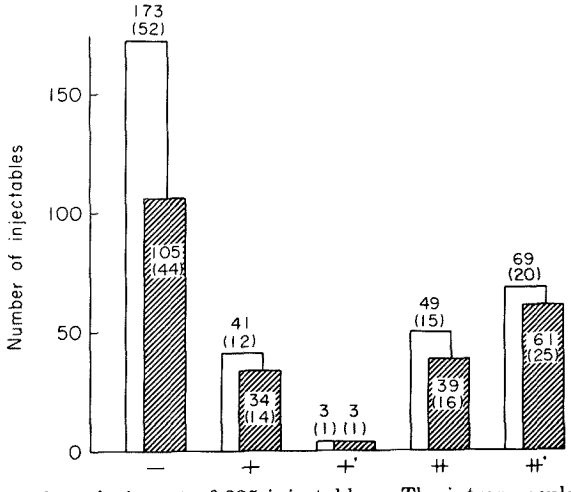

Fig. 3. Macroscopic hemolysis test of 335 injectables. The intramuscular injectables are presented with oblique lines. Percentages are given in the parentheses.

hemolysis test using ^{51}Cr-labeled RBC are shown in Table 1. A similar tendency to that by the macroscopic hemolysis test was seen. However, using this method, some injectables were gelatinized when mixed with blood, which disabled us dividing the mixture into sediment and supernatant.

Cytotoxic effect (CTE) on cultured cells

The results of CTE of 107 kinds of injectables are shown in Table 2. On the whole, CTE(—) was 16%, (+) 15%, (++) 34%, and (+++) 35%. About two-thirds (69%) of the injectables tested revealed more or less CTE. All of 19 injectable antipyretics and analgesics, and many of the antibiotics and antiallergic agents

TABLE 1. *Rate of hemolysis with ^{51}Cr-labeled RBC*

Injectables	Rate of hemolysis				Total
	0–9%	10–49%	50–74%	75–100%	
Antipyretics & analgesics	4	2	1	13	20
Antibiotics	16	3	4	13	36
Antiallergic agents	4	4	2	3	13
Vitamins	9	2	4	2	17
Others	14	2	3	4	23
Total	47	13	14	35	109

TABLE 2. *The results of cytotoxic effect of 107 injectables*

Injectables	Cytotoxic effect				Total
	−	+	++	+++	
Antipyretics & analgesics	0	0	3	16	19
Antibiotics	3	8	14	12	37
Antiallergic agents	2	2	4	4	12
Vitamins	5	6	7	1	19
Others	7	0	8	5	20
Total	17	16	36	38	107

manifested CTE of (++)~(+++). On the other hand, many of the injectable vitamins and other injectables showed (—)~(+), in which no CTE occurred.

Muscle lesion due to injection

Short-term observation. Not only in cases of normal saline but also in cases of injectables, the trace of needle puncture on the fascia, and discontinuity of the muscle fibers, slight bleeding and cellular infiltration were often observed macroscopically and microscopically, respectively, on the 3rd day of injection. However, these findings usually disappeared on the 7th day. On the other hand, in the case of 6% acetic acid solution as the control of (+++), a broad necrosis of the muscle, marked bleeding and cellular infiltration in the surrounding of the injection site were observed both on the 3rd and on the 7th day of injection. The data were adopted only when there were found similar autopsy findings in two rabbits which received injection of 0.5 ml of the same injectable. In general, the lesions were less remarkable on the 7th day than on the 3rd day, but severe lesions remained on the 7th day in cases where those were present on the 3rd day (Figs. 4, 5).

As a whole, 27% of the injectables tested showed no evidence of muscle lesion on the 7th day, and 51% showed severe damages to the muscle. All of 16 antipyretics and analgesics, about two-thirds of 31 antibiotics and about a half of antiallergic agents caused severe damages, while injectable vitamins and others caused no or slight damages (Table 3).

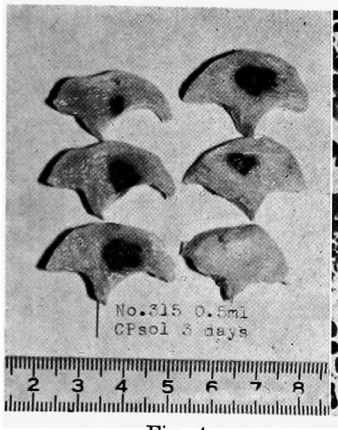

Fig. 4.

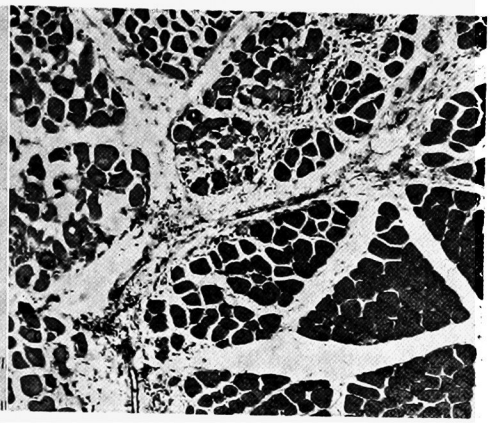

Fig. 5.

Fig. 4. Macroscopic finding of CPsol, 0.5 ml i.m. after 3 days. Marked necrosis with discolored change.

Fig. 5. Microscopic finding of CPsol, 0.5 ml i.m. after 3 days. Marked necrosis, cellular infiltration and hemorrhage. Hematoxylin and eosin. × 32

TABLE 3. *Grades of muscle lesion by the kinds of injectables*

Injectables	Muscle lesion										Total
	3rd day					7th day					
	−	+	++	+++	++++	−	+	++	+++	++++	
Antipyretics & analgesics	0	0	0	0	16	0	0	0	1	15	16
Antibiotics	1	5	3	1	21	7	2	2	2	18	31
Antiallergic agents	0	4	1	1	4	5	0	0	1	4	10
Vitamins	2	12	2	0	1	6	10	0	0	1	17
Others	2	5	2	2	4	6	2	4	0	3	15
Total	5	26	8	4	46	24	14	6	4	41	89

Long-term observation. Marked fibrosis and fatty infiltration were observed in the vastus lateralis muscle of three rabbits which had received a single intramuscular injection of 0.5 ml of Chloramphenicol-sol one month prior to sacrifice (Fig. 6).

In cases of injections of 0.5 ml three times a week for 4 consecutive weeks, 25% sulpyrine preparation, Chloramphenicol-sol and 1% Vena (diphenhydramine hydrochloride), which are of strong hemolytic potential, gave marked fibrosis, and Neophyllin M (diprophylline), which is of moderate hemolytic potential, gave moderate fibrosis one month after the last injection. In contrast to the above injectables, normal saline and Cobamamide, a co-enzyme B_{12} preparation which is negative in hemolysis, gave no significant change (Fig. 7).

Further, in the case of daily injection of 0.5 ml of 25% sulpyrine preparation for 5 consecutive days, marked fibrosis was observed 3, 6 and 12 months later. In

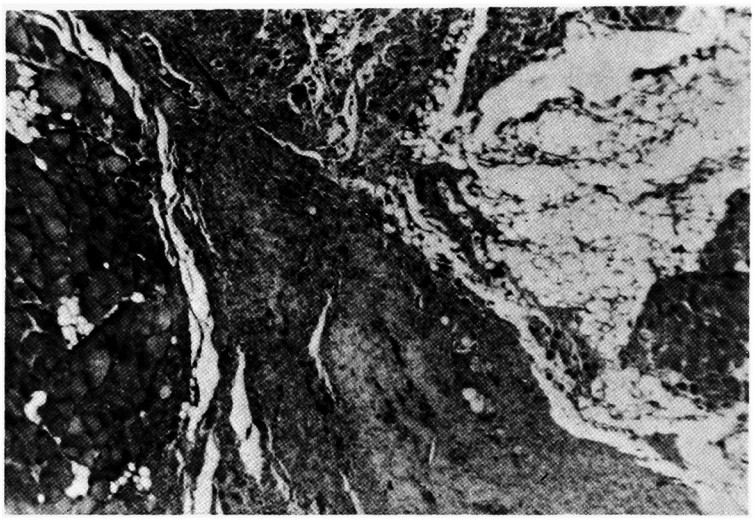

Fig. 6. Microscopic finding of vastus lateralis muscle at 28 days after 0.5 ml injection of CPsol. Fibrosis and fatty infiltration are prominent. Hematoxylin and eosin. ×40

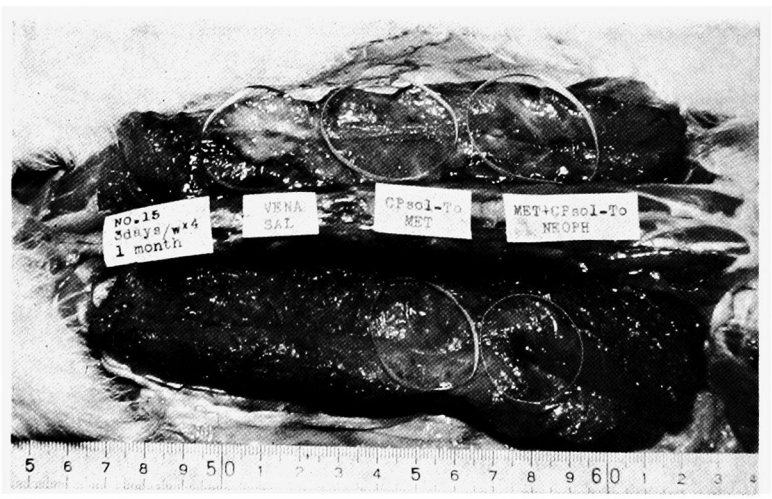

Fig. 7. Macroscopic finding of the sacrospinalis muscle at 1 month after 12 injections. In the lower, from left to right, normal saline, 25% sulpyrine, Neophyllin M. In the upper, 1% Vena, CPsol, sulpyrine+CPsol.

the case of the mixture of 0.25 ml of 25% sulpyrine preparation and 0.25 ml of CP-sol, more marked fibrosis was observed after the same period as mentioned above (Fig. 8).

Correlations among muscle lesion, hemolysis, CTE and physico-chemical properties of injectables

Thirty-four (81%) of 42 injectables which showed hemolysis of (—) ~(+) caused

Fig. 8. Macroscopic finding of sacrospinalis muscle at longterm observation. Five injections with sulpyrine and sulpyrine+CPsol. In the lower, 25% sulpyrine, upper sulpyrine+CPsol. From left to right, 3 months, 6 months and 12 months later.

muscle lesion of (—)~(+), and 43 (91%) of 47 injectables which showed hemolysis of (±)~(±)' caused severe muscle damages. On the other hand, only one of 51 injectables which showed muscle damages of (±)~(±±) caused CTE of (—), and out of 38 injectables which caused muscle damages of (—)~(+), 25 showed CTE of (—)~(+), but 13 showed CTE of (±)~(±±).

No significant correlation was found between pH of injectables and muscle lesion as far as pHs between 3 and 10 were concerned. But, most of the injectables with osmotic ratio over 5 caused severe muscle lesions.

The results of our studies on pH, osmotic ratio, hemolytic potential, CTE and muscle-damaging potential of some of the injectables tested are summarized in Table 4.

Discussion

In Japan, the first case of the quadriceps femoris muscle contracture was reported by Morisaki in 1945, but the etiology was not elucidated (Morisaki 1958). In the next year, Itoh suggested that this seemed to be caused by injections. Thereafter, many cases of muscle contracture in children were reported mainly in the field of orthopaedic surgery.

Intramuscular injection for babies and infants has been indicated in Japan to give into the central part of the front thigh, while in Western countries the lateral thigh is indicated in most of the medical reports and nursing textbooks (Turner 1920; Augustine et al. 1952; Gilles and French 1961; Culver 1969). Besides, the deltoid or gluteal muscles are commonly used in cases of adults and infants. As a result, not a few cases of muscle contracture have been reported in several countries (Gunn

TABLE 4-1. *Antipyretics and analgesics*

Injectables		Injection method	pH	Osmotic ratio	Hemolytic potential	Cytotoxic effect	Muscle lesion	
							3rd day	7th day
Metilon	10% 2ml	im. hd. iv.	7.1	2.5	++′	++	+++	++
Metilon	25% 2ml	im. hd. iv.	7.0	5.8	++′	++	+++	+++
Metilon	50% 1 ml	im. hd. iv.	6.9	10.0	++′	++	+++	+++
Avapyra	3 ml	im. iv.	5.3	4.9	++′	++	+++	+++
Pentagin 30	30 mg/1 ml	im. hd. iv.	4.3	1.1	++′	++	+++	+++
Sosegon	30 mg/1 ml	im. hd. iv.	4.3	1.3	++′	++	+++	+++
Irgapyrin	3 ml	im.	10.1	5.1	++′	++	+++	+++
Grelan	2 ml	im. iv.	5.9	10.1	++′	++	+++	+++
NA*	1 ml	im. hd.	7.2	7.0	++′	++	+++	+++
Obelon*	1 ml	im. hd.	9.2	5.3	++′	++	+++	+++
LL*	1 ml	im. hd.	5.9	6.9	++′	++	+++	+++
Asdrin-S*	1 ml	im. hd.	6.2	7.9	++′	++	+++	+++
Asdrin-S: 2*	2 ml	im. hd.	6.5	5.3	++′	++	+++	+++
C-Noblon†	2 ml	im.	6.3	7.9	++′	++	+++	+++
Noblon A†	2 ml	im.	6.7	7.0	++′	++	+++	+++
20% Phenobal†	200 mg/1 ml	im. hd.	8.7	—	++′	++	+++	+++

* Remedies against common cold; † Hypnotics and sedatives.

1964; Lloyd-Roberts and Thomas 1964; Hagen 1968), particularly in Japan where injections are routinely administered (Sato et al. 1965; Negishi et al. 1970; Sakurai 1972; Sato and Sano 1976).

The etiology of these muscle contractures had been thought to be congenital (Hněvkovsky 1961; Fairbank and Barret 1961), inflammatory or traumatic. However, it has been suggested by several investigators that most cases of muscle contracture were due to injections (Gunn 1964; Hagen 1968; Negishi et al. 1970). Since such a sequela ensues from the process of fibrosis in muscle, no serious symptoms develop during the course of injections, but they become manifest several months, or one to two years after injections. In any way, it is obvious that there are problems in injectables themselves.

According to the Japanese pharmacopoeia, it is indicated that the injectables of low pH irritate tissues and give severe pains, and those of high pH may cause tissue erosion; therefore, pH of injectables is desirable to be as nearly neutral as possible. And, pH of human plasma is known to average about 7.4 ± 0.05. Nevertheless, pH of 304 kinds of injectables tested in the present study ranged unexpectedly from 1.4 to 12.8. There may be pharmaceutical reasons. However, taking it into consideration that the injectables are to be used for human, it ranges too widely.

The pharmacopoeia indicates also that the osmotic pressure of injectables should be close to that of plasma, because the cell membrane is semipermeable. However, our present study revealed that the osmotic ratio of 300 kinds of injectables showed unexpectedly a wide range from 0.2 to about 36, although there were many injectables with the osmotic ratio of about 1.

Next, there have been several reports on hemolysis of injectables (Mori 1953; Grosicki and Husa 1954; Okano and Matsuo 1957; Hammarlund and Pedersen-

TABLE 4–2. *Antibiotics*

Injectables		Injection method	pH	Osmotic ratio	Hemolytic potential	Cytotoxic effect	Muscle lesion	
							3rd day	7th day
Pc G 'B'	10^6 I.U./3 ml	im. iv.	6.5	3.4	++	++	+++	++
Viccillin	500 mg/3 ml	im.	9.0	2.7	−	+	+++	++
Natacillin	0.5 g/2 ml	im.	8.3	3.2	++′	++	++	++
Geopen	1 g/1.5 ml	im. iv.	6.7	11.8	++′	++	+++	++
Gripenin	1 g/1.5 ml	im. iv.	6.7	12.2	++′	++	+++	+++
Lilacillin	1 g/3 ml	im.	5.7	3.4	+	++	+++	+++
Staphcillin-V	250 mg/2 ml	im.	6.0	2.4	++	++	+++	+++
Colistin-M 'B'	10^6 I.U./2 ml	im. hd.	6.9	0.9	−	++	+	−
Colimycin 'painless'	10^6 I.U./2 ml	im. hd,	6.9	0.7	−	++	+	−
Gentacin	40 mg/1 ml	im.	5.0	1.1	++	++	+++	+++
Streptomycin sulfate	1 g/3.2 ml	im.	5.5	1.8	+	++	++	−
Kanamycin sulfate 'B'	1 g/3.2 ml	im.	7.1	4.1		+	++	+
Kanendomycin	200 mg/2 ml	im.	7.0	1.1	−	+	++	−
Lincocin	300 mg/1 ml	im. iv.	5.2	5.5	++′	++	+++	+++
Vistamycin	1 g/3 ml	im.	7.6	2.0	−	−	+	−
Chloromycetin sol	1 g/4 ml	im.	6.8	2.4	++	+++	+++	+++
CP sol 'T'	1 g/4 ml	im.	6.9	1.0	++	+++	+++	+++
Kemicetine	1 g/4 ml	im.	6.8	1.0	+	++	+++	+++
Paraxin sol	1 g/4 ml	im.	6.1	1 2	+	++	+++	+++
Myclocin sol	1 g/4 ml	im.	6.4	2.1	++	++	+++	+++
Chloromycetin succinate	25%	im. hd. iv.	6.5	4.4	++′	++	+++	++
Paraxin succinate	25%	im. hd. iv.	6.2	4.5	++′	+++	+++	+++
Paraxin succinate A	20%	im. hd. iv.	6.7	3.3	++′	++	+++	+++
Terramycin	50 mg/1 ml	im.	8.8	35.7	++′	+++	+++	+++
Ossitetra L	50 mg/1 ml	im.	8.3	35.8	++′	+++	+++	+++
Pyrrocycline-N	300 mg/2 ml	im.	3.2	7.0	++′	++	+++	+++
Keflodin	1 g/s 4 ml	im.	5.1	2.2	−	+	−	−
Ceporan	1 g/s 4 ml	im. iv.	5.0	2.1	−	+	+	−
Keflin	1 g/4 ml	im. iv.	5.1	3.5	++′	++	+++	+++
Cefamezin	0.5 g/2 ml	im.	5.6	2.2	−	+	+	+
Kanacillin 'B'	0.5 g/2 ml	im.	6.9	—	+	++	+++	+++

TABLE 4–3. *Antiallergic agents*

Injectables		Injection method	pH	Osmotic ratio	Hemolytic potential	Cytotoxic effect	Muscle lesion	
							3rd day	7th day
Vena	1% 1 ml	im. hd.	4.4	1.0	+	++	+++	+++
Vena	3% 1 ml	im. hd.	4.0	1.1	++	++	+++	+++
Resmin	1% 1 ml	im. hd.	6.2	1.6	++	++	+++	+++
Resmin	1.5% 2 ml	im. hd.	5.7	1.6	++	++	+++	+++
Allergin	5 mg/1 ml	im. hd. iv.	6.5	1.1	+	+	+	−
Bisuonin	2 ml	im. hd. iv.	5 2	1.4	+′	+	+	−
Entra	0.2% 1 ml	im. hd.	5.9	3.3	−	−	++	−
Alimezine	0.25% 1 ml	im. hd. iv.	6.4	1.0	++	++	+++	+++
Bellphagen H	2 ml	hd. iv.	7.7	2.2	−	−	+	−
Neo-minophagen C	2 ml	hd. iv.	6.9	3.7	−	++	+	−

TABLE 4–4. *Vitamins*

Injectables		Injection method	pH	Osmotic ratio	Hemolytic potential	Cytotoxic effect	Muscle lesion 3rd day	Muscle lesion 7th day
Chocola A	5×10^4 I.U./1 ml	im.	4.5	1.0	−	++	−	−
Ricketon	3×10^5 I.U./0.5 ml	im.	5.5	—			+	
Coxylase	50 mg/2 ml	im. hd. iv.	6.4	1.7	−	−	+	+
Bisulase	20 mg/2 ml	im. hd. iv.	5.6	1.3	−	+	+	+
Flavitan 10	10 mg/1 ml	im. hd. iv.	5.6	1.2	−	−	−	−
Hexermin-P	10 mg/1 ml	im. hd. iv.	6.5	1.8	−	++	+	−
Pyromijin 30	30 mg/1 ml	im. hd. iv.	6.9	2.2	−	+	+	+
Funacomide 500	500 μg/2 ml	im.	6 4	1.2	−	−	+	+
Redisol-H	1000 μg/1 ml	im.	4.4	1.0	++	++	+	+
Redisol	1000 μg/1 ml	im.	6.0	1.0	−	++	+	+
Calomide-Me	500 μg/1 ml	im. hd.	6.7	1.1	−	+	+	−
Viscorin	100 mg/1 ml	im. hd. iv.	6.8	4.2	++′	++	++	+
Vitamin C 'F'	100 mg/1 ml	im. hd. iv.	6.7	4.9	++′	++	+	+
Vitacimin	100 mg/1 ml	im. hd. iv.	7.1	4.4	+′	++	++	+
Kaywan 10	10 mg/1 ml	im. hd. iv.	5.6	3.2	+	+	+	−
Kaytwo 10	10 mg/1 ml	im. iv.	6.0	3.5	−	−	+	+
C-para	2 ml	im. hd. iv.	5.1	2.1	+	++	+++	+++

TABLE 4–5. *Other injectables*

Injectables		Injection method	pH	Osmotic ratio	Hemolytic potential	Cytotoxic effect	Muscle lesion 3rd day	Muscle lesion 7th day
Contomin	10 mg/2 ml	im. iv.	5.5	0.9	++	+++	+++	+++
Cercine	10 mg/2 ml	im. iv.	6.5	—	++′	+++	+++	+++
Buscopan	20 mg/1 ml	im. hd. iv.	4.3	1.0	−	−	−	−
Vitacampher	0.5% 1 ml	hd. iv.	3.9	0.2	++	++	++	++
Neophyllin M	0.3 g/2 ml	im. hd. iv.	6.9	1.5	++	++	+++	++
Funasol	10 mg/1 ml	im. hd.	6.1	1.3	−	−	+	−
Esberiven	2 ml	im. iv.	7.5	5.6	+	−	+	−
Theo-esberiven	2 ml	im. iv.	7.1	7.0	+	++	+++	+++
Theraptique	2 ml	im.	4.1	1.6	++	+++	++	++
Inolin	0.1 mg/1 ml	im. hd.	5.0	1.0	−	−	+	−
Thmermon 'K'	1 ml	hd.	4.9	1.3	++	++	+++	++
Venostasin	150 mg/5 ml	iv.	5.4	1.9	++	++	+	+
Neomyson G	1 g/20 ml	im. iv.	3.6	0.8	−	++	++	+
Solco hepsyl	100 mg/2 ml	im.	6.7	1.7	−	−	−	−
Solco seryl	2 ml	im. iv.	6.9	3.6	−	−	+	−

Bjergaard 1961; Fievet et al. 1971). However, they were all concerned with intravenous injection. For example, Mori (1953), who added 1 ml of injectable into the mixture of 10 ml of human blood and 90 ml of normal saline and incubated the mixture at 37°C for one hr, reported that Grelan, an antipyretic-analgesic consisting of pyrabital and aminopyrine, showed a complete hemolysis, and so it should be injected not intravenously, but intramuscularly.

In any case, it is obvious that they considered the intravascular hemolysis

due to injectables to be the major problem. The other authors mentioned above investigated this matter with the same or similar conception. On the contrary, we considered that injectables with hemolytic potential will damage more or less the muscle cells, since blood cells and muscle cells are similar in a broad sense of being cells, though they are morphologically and functionally different. The results of our macroscopic hemolysis test showed that injectables of severe hemolysis were 35%, and especially most of injectables for intramuscular use showed severe hemolysis.

Next, for quantitative analysis of hemolytic potential, the oxyhemoglobin method and the cyanmethemoglobin method were tried by means of spectrophotometry. In the former, the supernatant after centrifugation was diluted, then the optical density at the wavelengths of 542 nm and 576 nm was defined, and the hemolytic ratio was determined using distilled water as the control (100%). In this method, the hemolytic ratio was inaccurate in several injectables which denatured hemoglobin and changed the pattern of spectrum. In the latter, 0.2 ml of the supernatant was mixed with 4 ml reagent of the modified Drabkin method (0.2 g of $K_3Fe(CN)_6$ and 0.05 g of KCN in 0.01 M phosphate buffer, pH 7.0), then after 20 min the optical density at 540 nm was defined, and the hemolytic ratio was determined. But some injectables reacted with the reagent. Then, we tried the ^{51}Cr-labeled RBC method for quantitative hemolysis test.

The results of hemolysis tests demonstrated that there is a considerably close connection between the hemolytic potential of injectables and the severity of muscle lesions by them. Nishijima (1977) also reported that muscle contractures were experimentally observed one month after injections in cases of 70% of injectables with severe hemolytic potential. Therefore, the hemolysis test of injectables is considered to be useful at least as a screening test of muscle lesions due to injectables.

Next, we studied the cytotoxic effect (CTE) of injectables on cultured monolayer cells (Oshida et al. 1974). Umeda (1974) also studied the effect of some medical drugs on the cultured cells by the use of the panel method. However, this method may be too sensitive because of microscopic observations as compared with other methods, although it is useful for studying easily the effect of many injectables.

There are not a few reports on animal experiments in intramuscular injection. For example, from the observations in clinical practice concerning the variation in pain response following the injection of several broad-spectrum antibiotics, Hanson (1961) examined the local tissue changes resulting from the subcutaneous and intramuscular injection of several antibiotics, and revealed that chloramphenicol succinate and tetracycline produced the most severe necrosis, while oxytetracycline produced the least necrosis.

On the other hand, Shintani et al. (1967) reported a new method for gross evaluation of the local tissue irritation of drugs by intramuscular injection in rabbits, in which the local tissue irritation was grossly scored from 0 (no discernible

gross reaction) to 5 (wide-spread necrosis), and the irritation was graded into six categories; none, slight, mild, moderate, marked, and severe, by the average irritation score from two or more rabbits.

In either case, they recognized that some injectables may cause necrosis by intramuscular injection, but even Hanson, a competent clinical pathologist, did not come to think of the possibility of fibrosis from necrosis, much less from fibrosis to contracture.

Our results obtained 7 days after a single intramuscular injection of 89 injectables to rabbits revealed that severe muscle lesion including necrosis were observed in 45 cases (51%) at the seventh day. Most of the injectables which caused severe muscle lesion were antipyretics, analgesics, antibiotics and antiallergic agents.

On the other hand, long-term observation revealed marked fibrosis and fatty infiltration following one, five or twelve injections of CPsol and sulpyrine. Mitsuyasu et al. (1976) reported that muscle lesion could be repaired although there is some initial irritation. But, our observation elucidated that once the muscle was necrotized, irreversible fibrosis should occur though the size of lesion might be reduced to some extent after a long time.

The results of our pathohistological investigation of rabbit muscles injured experimentally by injection quite resembled those of the muscle contracture patients in increased fibrosis, scar tissue, fatty infiltration, and atrophy of muscle fiber (Sato et al. 1965; Hagen 1968; Yamaguchi et al. 1970; Tsubota et al. 1975), and further revealed that many injectables with strong hemolytic potential or strong CTE caused severe muscle lesions such as necrosis and fibrosis by intramuscular injections. Accordingly, it is evident that most cases of muscle contracture are sequelae of physiological incompatibility of intramuscular injectables.

It is considered that the particular structure of the muscle (bipennate or multipennate) and the degree, size or position of the scar and adhesion have a significant connection with the occurrence of the symptom of muscle contracture (Sato and Sano 1976).

It is sure that injectables with tissue-damaging potential may cause necrosis or fibrosis in the muscles such as the quadriceps femoris, deltoid or gluteal muscles. Therefore, injection of such injectables should be most carefully done.

In any case, it is a great matter for doctors in medical practice that they were not informed of either physico-chemical properties or tissue-damaging potential of injectables.

In conclusion, we would like to emphasize the followings:

1) Doctors should be informed of the physico-chemical properties, at least pH and osmotic ratio, and tissue-damaging potential of each injectable, in particular, for intramuscular use.

2) Pharmaceutical companies should exert all possible efforts to improve injectables.

3) Doctors should administer injection to a minimum and only in cases of actual need.

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