NOTES ON EXPERIMENTAL ANAPHYLAXIS INDUCED BY SIMPLE CHEMICAL COMPOUNDS (I)

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During the past two decades anaphylactic phenomena induced by simple chemical compounds have been studied experimentally by a few investigators, especially by Landsteiner and Sulzberger and their collaborators in the United States of America. We also have carried out several experiments along this line, and our observations conducted in this laboratory are briefly described herein without referring to the reports of other workers, since we have been out of touch with foreign literatures for the past few years.

Principle of Experimental Methods

As a rule, guinea pigs weighing 200g to 300g were used, at least, five of them being employed for each part of an experiment. Various substances were applied as sensitizing and reaction-provoking agents in the manner described below. Hypersensitivity was examined by testing shock symptoms, the intestinal contraction and the local skin reaction: the shock was incited by injecting a provocative dose intravenously, and its severity was determined by general symptoms, the fall of body temperature and macroscopic changes of the lung; the intestinal contraction was tested by Schultz-Dale technique with the segment of small intestine suspended in 50 cc of Tyrode solution, to which a provocative dose was added; the skin reaction was instigated by intracutaneous injection of a definite amount of provocative agent, and the intensity and the size of the erythematous lesion were determined 24 hours after the injection. In this skin test, the abdominal area was usually used, the hair having been carefully clipped off beforehand, and in most cases injections were given in two to four separate sites of the abdomen of each animal to compare reactions to the same dose or to detect reactions to different substances or to various dilutions of the same chemical. Provocative doses for those were determined by the results of preliminary tests; general, intestinal and skin reactions of normal guinea pigs to each chemical were carefully examined, and the amounts which did not incite any noticeable change were fixed as maximal doses for testing hypersensitivity. Readings of the results of skin reactions were usually made 24 hours after injecting the provocative dose. In the case of active sensitization, those tests were tried two to three weeks after the last administration of a sensitizing material, and in the case of passive sensitization, which was accomplished by injecting a certain amount of the serum or serum protein obtained from the actively sensitized guinea pig into the jugular vein of normal animals, hypersensitivity was tested 24 hours after the transfer, unless otherwise stated. When rabbits, rats or mice were used, sensitization and detection of hypersensitive response were conducted in the similar way. More descriptions relating to special experimental procedures will be given as the occasion demands.
Sensitizing Capacity of Various Chemicals

*Arsenious acid* Anaphylactogenic capacity of arsensive acid and other arsenic compounds, such as atoxyl sodium arsenate and Neo-neo-arsenin (C₆H₃As(OH)NHCH₂OSONa), was investigated by Y. Yamasaki. In the experiments for active sensitization with arsensive acid, the chemical was administered as follows: a) 0.1cc of the 0.01% or 0.5% saline solution was given via the intracutaneous route once a day for 7 to 10 days successively, b) 0.5cc of the 0.5% solution was injected daily for 5 days through the intraperitoneal route, c) a single dose of 1.0cc of the 0.5% solution was given, intravenously or d) 1g of the mixture of arsensive acid and glucose (1:9) was given daily through the oral route for 12 successive days. Skin reactions tested by intracutaneous injections of 0.1cc of 0.25% arsensive acid, 0.1cc of 2% atoxyl, 0.1cc of 1% sodium arsenate and 0.3cc of 0.3% Neo-neo-arsenin showed that the guinea pigs, which had been treated in these ways, manifested definite hypersensitive reactions to arsensive acid but not to other arsenic compounds, with an exception of a few cases of slight reactions to Neo-neo-arsenin, and evidence was directed to the intracutaneous route as the most effective way of sensitization. But guinea pigs thus sensitized did not show, to a doubtless extent, anaphylactic shock or hypersensitive intestinal contraction to arsensive acid, when the former was tested with 0.5cc of 0.0125% solution and the latter with 1.0cc of 0.005% solution.

An injection of 0.5cc of 0.015% solution of arsensive acid into the non-treated normal guinea pigs was found to incite anaphylactoid phenomenon, which was verified by detection of changes of body temperature, complement titer, the lung, coagulation time of the blood and general symptoms. By comparing such changes with those observed in the sensitized guinea pigs, it was concluded that true anaphylactic shock did not occur. Attempts to sensitize actively by 10 daily subcutaneous injections of 0.1cc of 10% solution of atoxyl or 1% solution of sodium arsenate failed, while similar injections of 3% solution of Neo-neo-arsenin seemed to sensitize, judging from the positive skin reaction to the sensitogen noticed in some cases. Passive sensitization was tried with the serum proteins of the guinea pigs allergized actively by intracutaneous injections of arsensive acid. One cc of the serum or of its albumin, euglobulin or pseudoglobulin was injected intravenously; these serum protein solutions were prepared by the method described by Felton and Swineford. The skin reaction to arsensive acid revealed that allergic state was passively established by means of the serum and the globulins, but the albumin fraction was unable to transfer hypersensitivity.

*Acetyl-salicylic acid* A series of experiments was carried out by M. Suzuki with three preparations of acetyl-salicylic acid, namely, aspirin (Japanese pharmacopoeia), aspirin (Bayer) and soluble aspirin. Sensitization was attempted by giving 0.1% solution of the drug through various routes in different quantities, a) 0.5cc was given daily for 10 days through the intracutaneous route, b) 1.7cc was injected three times (every second day) through the intraperitoneal route, c) a single dose of 0.7cc per 100g body weight was applied through the intravenous route, d) 1.0cc was slowly administered through the
oral route once a day for 10 days, e) the mixture of the aspirin solution and horse serum (5%) was used as in a). Anaphylactic reactions were examined with 0.1% solutions by injecting 0.5cc in the skin test, by adding 0.5cc to Tyrode solution in the intestinal test and by injecting 0.5cc per 100g body weight in the shock test. The skin test revealed definite erythematous reactions in guinea pigs sensitized by the procedure e), but dubious results in those treated by other procedures. Thus, it was shown that on addition of horse serum accelerated sensitizing capacity of aspirin. Passively sensitized animals by the injection of the serum of actively sensitized ones showed similar results. The Dale test and the shock test did not give hypersensitive reaction, though, in some cases shock and intestinal reactions which appeared to be anaphylactic took place.

**Barbital** It was found by K. Hashimoto that Barbital, Barbitalum soluble, Phenobarbital and soluble Phenobarbital produced hypersensitive skin reactions, when guinea pigs were sensitized with the mixture of one of these substances and horse serum. In the experiments with soluble Barbital (C$_7$H$_6$N$_2$C$_4$H$_2$O$_3$Na 3% solution) was used for the purpose of active sensitization in the following manner: a) an intracutaneous injection of 0.5cc of the pure solution or b) of the mixture of the solution and horse serum (the serum was added to 5%) was given daily for 10 days; c) three intraperitoneal injections of 0.5cc of the mixture were given with the interval of 48 hours; d) a single dose of 0.5cc of the mixture was injected intravenously. The skin reaction was tested with 0.5cc of the 3% solution, and the intestinal reaction was examined with 1.0cc of 2% solution. Some of each group of guinea pigs which were treated with the mixture of Barbital and horse serum showed hypersensitive skin reactions. Results of the Dale test were not definite. Phenobarbital and soluble phenobarbital showed similar results.

**Mercury** Experiments with mercury and mercuric chloride were carried out by Y. Yamasaki. Active sensitization was tried in two ways: a) mercury ointment was prepared by mixing mercury (Caulk) and lanolin at the ratio of 1:5, and 2.0g of it was smeared on the back once a day for 10 successive days, changing the site of application each time; b) 1.0g of the mixture of mercury and lactose (mixed at the ratio of 1:100) was given with the ordinary food once a day for 10 successive days. The skin reaction was tested by rubbing 2.0g of 10% mercury-lanolin ointment or by injection intracutaneously 0.1cc of 0.0005% mercuric chloride solution. The intracutaneous test showed, in animals sensitized in either way, definitely hypersensitive reactions of various degrees of intensity, but in response to the ointment no positive reaction occurred. Guinea pigs injected with 1.0cc of the serum obtained from actively sensitized animals manifested similar skin reactions to HgCl$_2$.

**Sulfonamides** Attempts have been made to induce anaphylactic phenomena with several preparations of sulfonamides, but failed so far. B. Imao and H. Mikami experimented with Prontosil soluble (Bayer), Gerison (p-aminophenylsulfonamide) and Therapal (p-aminobenzencesulfonamide). They tried to sensitize by giving daily an intracutaneous dose of $0.2cc$ or an intramuscular dose of $0.5cc$ of pure solutions or of the mixture of Prontosil and horse serum (9:1) for successive 10 days. And 2 weeks later, the skin,
Dale and shock tests were conducted, but even in the skin test there appeared no hyper-sensitivity. Following them, G. Miyaoa also studied the sensitizing capacity of ‘Albasil’ (4-(4′-aminobenzenesulfonylamide)-benzenesulfone-dimethylamide), ‘Region’ (p-aminobenzeNsulfoneacetamide) and ‘Adiplon’ (2-(p-aminobenzenesulfonylamide)-pyridine), by applying these drugs as pure solution or as the mixture with horse serum through intracutaneous, intramuscular or intraperitoneal route. Results of these experiments were all negative.

**Picric acid** Y. Ikuta allergized guinea pigs with picric acid and examined the reactions to picric acid, potassium picrate and sodium picrate. Active sensitization was carried out by intracutaneous injection or by anointment of the sensitigen. In the former case, 0.95cc of 0.5% or 1.0% solution or of the mixture of picric acid and horse serum, which was prepared by adding the serum to 1% aqueous solution of the chemical at the ratio of 5:5, 1:4, or 1:9, was given daily for 10 days. And in the latter case, 1g of the ointment composed of 5.0g of picric acid, 10cc of horse serum and 100g of lanolin was applied on the skin once a day for 20 days, changing the site each time to avoid erosion. The skin reaction was tested with 0.25cc of 0.1% solution, the intestinal reaction with 0.5cc of 0.1% solution and the shock with 0.25cc of 0.5% solution per 100g of body weight. Hypersensitive skin reactions were incited in guinea pigs injected with the mixtures; the ratio of 9 (picric acid solution) to 1 (horse serum) seemed to be the most potent. Positive reactions were given also by picrates, though not so marked as by picric acid. But allergic response was not definitely shown in shock and Dale tests. In passive sensitization, 1.0cc of the serum of actively sensitized guinea pigs or various amounts of its protein fractions prepared by the method mentioned above2 were injected. The serum and its euglobulin and pseudoglobulin fractions were able to allergize, which was proved by the positive skin reaction, but its albumin fraction could not transfer hypersensitivity.

**Laxatol** Using colloidal solution of Laxatol (isovalerylacetylphenolphthalein) prepared by Weimarn's method, S. Makihata intended to induce anaphylaxis. Active sensitization was attempted through intracutaneous and oral routes with approximately 1% Laxatol solution; an intracutaneous dose of 1.0cc or an oral dose of 2.0cc was administered once a day for 10 successive days. And, the skin reaction was detected with 0.1cc of 0.5% solution, the intestinal reaction with 0.4cc of 1% solution, and the shock with 5.0cc of 0.5% solution. Allergic skin reaction, not so marked but definite, was produced in some of the guinea pigs. Passive sensitization was accomplished by an injection of 5.0cc of the serum of actively sensitised one. Shock and Dale tests gave negative results in both cases of sensitization.

**Rivanol** Anaphylactogenic ability of Rivanol (Bayer) C14H2104N3 was investigated by H. Yamasaki. Several sensitizing agents made with this derivative of acridine were employed, i. e., a) 1:500 aqueous solution, b) 1:500 solution to which horse serum was added to 0.1%, c) 1:500 solution to which horse serum was added to 1.0%, and d) the mixture of 5.0g of Rivanol, 10cc of horse serum and 100g of lanolin. In active sensitization, 10 daily doses were administered through various routes: 0.25cc of a), b) or c) agent through the intracutaneous route; 2.0cc of a), b) or c) agent per 100g body
weight through the vein; 0.5g of d) agent by anointing, and 1.0cc of a) or c) agent via oral route; 0.5cc of a) or c) agent per 100g body weight through the intraperitoneal route. Skin reaction, shock and intestinal contraction were tested with 0.1cc of 1:10,000 solution 0.5 cc of 1:10,000 solution per 100g body weight and 0.5 cc of 1:1,000 solution respectively. Those treated with the mixture of Rivanol and horse serum through intradermal, intraperitoneal or oral route manifested definitely allergic skin reaction, while others injected with pure solution showed doubtful reaction occasionally. And sensitization by anointment did not show any abnormal reaction either to intracutaneous injection of the above mentioned dose or to smearing of 15% or 25% Rivanol ointment. Shock and Dale tests did not reveal anaphylactic response, though there were some cases of uncertain results. Passive sensitization was established when 2.0 cc per 100g weight of the serum, its euglobulin or pseudoglobulin fraction, which was obtained from guinea pigs actively sensitized with the mixture through intradermal route was injected, as judged, from allergic skin reactions. Albumin fraction of the serum could not convey the hypersensitivity.

**Chromates** In Y. Sakurayama's experiments, potassium bichromate was used as sensitigen, and anaphylaxis was examined not only with the bichromate but also with potassium chromate, chrome alum and chromium carbonate. He tried to sensitize actively by 10 daily intracutaneous injections of 0.1 cc of 0.5% aqueous solution of the bichromate or of the mixture of the solution and horse serum (9:1), or by 20 daily inunctions of 1.0g of the ointment made of 1.0g of potassium bichromate, 10cc of horse serum and 100g of lanolin. The skin reaction was tested with 0.1cc of 0.1% solutions of the four kinds of chromium compounds. One tenth cc of 0.5% bichromate solution was employed in the shock test, and 0.5cc of the same solution was dropped into the bath in Dale test. When sensitized by intracutaneous injections of the mixture or by anointment, guinea pigs showed distinctly allergic skin reactions to all of the provoking agents, four chromates reacting without notable differences. The serum, its euglobulin and pseudoglobulin of actively sensitized guinea pigs were able to allergize passively, when 3.0 cc of the serum or 5.0 cc of the protein fraction was injected, although the albumin fraction was practically unable to do so. These proteins were fractionated with sodium sulfate as mentioned above. All of the sensitized guinea pigs failed to exhibit anaphylactic response in shock and Dale tests.

**Cresol** H. Akaza and H. Nemoto experimented with ortho, meta and para cresols. By means of pure cresol solutions or cresol solutions containing 5% of horse serum, they tried to sensitize actively as follows: a) intracutaneously, a dose of 0.6cc (divided and injected in 3 places) of 1:500 solution was given daily for 10 days; b) intravaginally, 0.5 cc of 1:200 solution was applied through the vagina once a day for 10 days, keeping the solution in the vagina by closing its outlet with weak forceps for about an hour each time; and c) intraperitoneally, 3 doses of 2.0 cc of 1:200 solution were given every second day. Skin, shock and intestinal reactions were tested with 0.1 cc of 1:500 solution, 0.2 cc of 1:500 solution per 100g body weight, and 0.2 cc to 0.3 cc of 1:5,000 solution, respectively. Some of the guinea pigs treated with the mixture of para-cresol and horse
serum appeared to show a slightly allergic skin reaction to para-cresol, but not to other cresols. Similar reactions against para-cresol were noticed in those injected with the serum of this group. Of interest in this connection is the fact that para-cresol is known to be the most poisonous of the isomers. Neither hypersensitive shock nor intestinal contraction occurred.

Formaldehyde Anaphylaxis instigated by formaldehyde was reported by Y. Yamasaki and H. Yamasaki. In active sensitization, they administered pure solutions of formalin which contained 35% of formaldehyde or formalin solutions mixed with 10% of horse serum through intracutaneous and intravenous routes. In the former case, a dose of 0.2 cc of 1:350 formalin solution or its mixture with horse serum was given daily for 8 days, and in the latter case, 0.5 cc per 100 g body weight of 1:300 formalin solution or its mixture with horse serum. The skin reaction was tested with 0.2 cc of 1:300, 1:800 and 1:1,000 formalin solutions. In the shock test 0.5 cc per 100 g body weight of 1:800 solution was injected, and in the Dale test 1.0 cc of 1:800 solution was used. Guinea pigs thus treated showed a distinct hypersensitive skin reaction; a tendency was noticed that more marked reactions took place in the intracutaneously sensitized group than in the intravenously sensitized group. An injection of 1.0 cc to 2.0 cc of the serum, its euglobulin or pseudoglobulin obtained from such guinea pigs passively allergized and gave positive skin reactions. The albumin fraction was devoid of this ability. Anaphylactic response was not observed in shock and intestinal tests.

Bismuth compounds To find out if bismuth compounds can induce anaphylactic phenomenon, H. Yoshinaga has conducted experiments with two Japanese commercial preparations; Rabis C₆H₅(OH)CO₂BiO and Bismutin C₄H₃O₂Na₂Bi₂ (0.01 g of Bi is contained in 1.0 cc of both preparations). A dose of 0.2 cc of the compound was intracutaneously injected daily for 10 successive days, and skin reaction was examined with 0.1 cc of the preparation diluted by 50 or 100 times. Guinea pigs treated with Rabis showed allergic reaction to Rabis and to a less degree to Bismutin while those injected with Bismutin did not respond hypersensitively. But when horse serum was added (5%), Bismutin could sensitize and brought out similar skin reactions. An injection of 2.0 cc per 100 g body weight of the serum taken from the guinea pig sensitized with the mixture of Rabis and horse serum allergized normal guinea pigs. Euglobulin and pseudoglobulin fractions of the serum, but not albumin, functioned in the same way as the serum in this respect.

Bromides Y. Tashiro experimented with potassium bromide and sodium bromide. He used in sensitization 5% and 10% pure solutions, and also these solutions containing 10% of swine serum; an intracutaneous dose of 0.2 cc or an oral dose of 1.0 cc of each solution was given once a day for 10 successive days. The skin reaction was tested with 0.1 cc of 0.1%, 0.5% or 1.0% bromide solution, and shock and intestinal reactions were examined with the application of 0.5 cc of 1% solution. In some guinea pigs treated with the mixture of the chemical and serum, slight allergic skin reactions were noticed. And the skin hypersensitive to one of the bromides was found to be hypersensitive to the other. Experiments of passive sensitization, which were carried out by injecting 2.0 cc of the serum per 100 g of body weight, resulted in showing that only the
serum of those sensitized with the mixture transferred hypersensitivity. Shock and Dale tests failed to illustrate abnormal reaction.

**Nickel compounds** Anaphylactogenic ability of nitrate and sulfate of nickel was investigated by K. Oshima. Active sensitisation was tried by 10 daily intracutaneous injections of 0.1 cc of 1.0% nickel solution containing 10% of horse serum, and by daily inunction for 10 days of ointment made of 1.0 g of the nickel compound, 10 cc of horse serum and 100 g of lanolin. The skin reaction, the shock and the intestinal contraction were tested with 1:1,000 solutions, using 0.1 cc, 0.25 cc per 100 g of body weight and 1.0 cc respectively. Distinct, but not marked, allergic reactions took place in the skin test; and two compounds reacted with each other. In some cases of shock and Dale tests there appeared reactions suggesting hypersensitivity, but results as a whole were not definite. Passive sensitization was successfully accomplished by injecting 1.5 cc per 100 g body weight of the serum or its protein fractions, except albumin, obtained from guinea pigs actively sensitized in either way; serum proteins were fractionated in the same manner as done by the other workers in this laboratory.

**Iodine** In search of anaphylactic reaction due to iodine, H. Yamasaki has carried out a series of experiments with Lugol's solution (Iodine 1, potassium iodide 1, distilled water 27), 0.5% iodine-glycerine solution and potassium iodide solution. In active sensitization 10% Lugol's solution, 0.5% iodine-glycerine solution and 1% aqueous solution of potassium iodide were used singly or in mixture with 10% of horse serum, and 0.2 cc of each agent was injected intracutaneously once a day for 10 days. The skin reaction was tested with 0.2 cc of 1:3,000 Lugol's solution, 1:300 dilution of the iodine-glycerine solution and 1% iodide solution. In the shock test, 0.5 cc per 100 g body weight of 1:50 Lugol's solution, 1:20 iodine-glycerine solution or 0.25% iodide solution was used, and in Dale test 1.0 cc of 1:100 Lugol's solution, 1:20 iodine-glycerine or 1% iodide solution was added to the bath. These dilutions were made with physiological saline solution. Hypersensitivity to the sensitizogen as well as to other iodine solutions was revealed in many cases of skin reactions, although potassium iodide did not give definite results, either as a sensitizing agent or as a provocative factor, and addition of horse serum to sensitizing doses seemed to have accelerated slightly theiranaphyl actogenic capacities. Marked hypersensitive intestinal contractions to Lugol's solution and iodine-glycerine solution were exhibited by those sensitized with the mixture of horse serum and Lugol's solution or iodine-glycerine. The shock test brought out like results, but hypersensitivity was not indicated so clearly. Passive sensitization was undertaken by injecting 0.5 cc to 2.0 cc of the serum or its proteins obtained from guinea pigs sensitized with the mixture of iodine and horse serum. Anti-Lugol and anti-iodine-glycerine sera and their globulin fractions were able, but anti-potassium iodide serum and albumin fractions of all sera were unable to transfer iodine hypersensitivity. And passively sensitized animals showed similar reactions to those of actively sensitized ones in skin, shock and Dale tests. In connection with this, he has observed on desensitisation phenomenon, which will be referred to later.

**Thymol** U. Hirano experimented with thymol C₆H₇·C₆H₅(OH)CH₃. By employing
1:1000 pure solution and a mixed solution with 10% of horse serum, active sensitization was tried through two routes, i.e., 0.2 cc was injected intracutaneously once a day for 10 days, or three doses of 1.0 cc per 100 g of body weight were intraperitoneally injected every second day. The skin reaction was tested with 0.1 cc of 1:5,000 or 1:6,000 thymol solution, and the shock was examined with 0.5 cc of 1:3,000 solution per 100 g of body weight while 0.5 cc of 1:1,000 solution was used in Dale test. Allergic erythematous reactions were induced in the skin test, but no abnormal change was noticed in shock and Dale tests. In passive sensitization experiments, it was found that the serum and its globulin fractions of guinea pigs sensitized through intracutaneous route with the thymol-serum mixture could transmit the hypersensitivity, when a dose of 0.5 to 2.0 cc was used. But its albumin fraction and serum of the guinea pig sensitized by intraperitoneal route failed to perform such function.

Nitro-Phenol A. Ehara and Y. Sakurai\(^2\) exposed that nitro-phenol sensitized guinea pigs and caused allergic skin reaction. To sensitize actively, they used aqueous solutions of ortho, meta and para nitrophenols and mixtures of these chemicals and horse serum or 1% of potassium alum, and applied them in two ways: a) a daily dose of 0.1 cc of 0.5% solution was injected intracutaneously 10 times; or b) three doses of 0.8 cc per 100 g body weight of 0.5% solution were given intraperitoneally every second day. Skin and intestinal reactions were tested with 0.5% solutions of nitro-phenols, 0.1 cc being used in the former and 0.5 cc in the latter. Shock was examined with a dose of 0.5 cc of 0.1% solution per 100 g of body weight. Hypersensitivity was shown in the skin test, but not in shock and Dale tests. Cross reactions were noticed among three isomers, though stronger reactions were induced in the sites where the sensitisogen, itself was injected as the provocative agent. There was a tendency that addition of horse serum accelerated sensitization, but apparently alum did not affect it. Passive sensitization was established by means of the serum of actively sensitized guinea pigs. Also in this case, hypersensitivity was illustrated only in the skin reaction.

Thyroxin K. Nomura and T. Yamasaki\(^2\) studied with Thyroxin (Rosche). They used 1% alkaline solution of the drug and the same solution containing 10% of horse serum, in active sensitization; 10 daily doses of 0.1 cc were given intracutaneously, or doses of 0.3 cc, 0.3 cc and 0.4 cc were injected intraperitoneally every second day. The skin reaction was tested with 0.1 cc of 0.2% pure solution, and the shocking dose was 0.5 cc of 1% solution per 100 g of body weight, while 1.0 cc of 1% solution was used in Dale test. Pure thyroxin solution failed to allergize, but many of the guinea pigs treated with the mixture of the drug and horse serum produced slight hypersensitive reactions. Shock and Dale tests gave negative results. Definite result was not shown, even in the skin test, by passive sensitization experiments, which were undertaken by injecting 2.0 cc per 100 g of body weight of the serum of actively sensitized animals.

Boric acid K. Nomura's\(^2\) experiments have shown that boric acid was unable to allergize. He used 5% aqueous solution and the same solution containing 10% of horse serum, and injected 0.1 cc intracutaneously once a day for 10 successive days. The skin reaction and the intestinal contraction were tested with 0.1 cc of 3% solution
and 1.0 cc of 5% solution, respectively. But, all tests turned out to be negative. Passive sensitization by the use of the serum was unsuccessful.

**Formic acid** In connection with his study on anaphylaxis induced by bee substances, M. Uyeyama has experimented with formic acid. He tried to sensitize intradermally by injecting 0.1 cc of 1% formic acid solution containing 10% of horse serum daily for 10 days. The skin test, which was conducted by 0.2 cc of 0.1% solution, brought out a faintly positive results in some cases.

**Benzene** Sensitization with benzene and nitro-benzene was attempted by M. Hara. These chemicals were diluted with sesame oil to approximately 10% concentration, and the solution was administered in three ways: a) 8 daily doses of 0.2 cc were given intracutaneously; b) 4 doses of 0.8 cc were injected subcutaneously every second day; or c) 8 daily doses of 0.8 cc were applied orally. The skin reaction was tested with 0.1 cc of 1:5.000 to 1:10.000 dilutions of the original oil solutions. Those sensitized through a) or b) route showed weak positive reactions, but orally treated ones gave a doubtful outcome. The reaction was relatively distinct when the chemical used in sensitization was applied as the provocative agent. Passively sensitized guinea pigs by the injection of 4.0 cc of the anti-benzene or anti-nitrobenzene serum or 5.0 cc of its globulins showed similar results; albumin, euglobulin and pseudoglobulin were fractionated from the serum by the method used by foregoing workers.

**Sensitization through other routes** With Lugol and protargol solutions, sensitizations through the urinary bladder and the nose have been accomplished. T. Yamasaki applied one dose of 0.5 cc of 1:6 Lugol's solution containing 10% of horse serum into the bladder with an urethral catheter of proper length (cut short for this purpose); urine in the bladder was pressed out beforehand, and after the solution was injected the outlet of the catheter was closed to keep the solution for a while. At first he tried to give several doses, but most of the animals died during sensitization, and finally it was decided to sensitize with a single dose. Intestinal reaction was examined with 1.0 cc of 1:100 Lugol's solution. Allergic contraction was noticed in 2 cases out of 5. And dissection showed inflammation in side of the bladder of the guinea pig which reacted positively in Dale test. Simultaneously, he made an experiment with egg albumin likewise, and succeeded in sensitization through the bladder, which was proved by intestinal contraction of the albumin. M. Takano conducted sensitization through the nasal route. He applied 10% Lugol's solution or 3% protargol solution with cotton swabs once a day for 10 days. The skin reaction was tested with 0.2 cc of 1:500 Lugol's solution and of 3% protargol solution. Slight hypersensitive reactions to these chemicals appeared.

Thus, it has been found that various chemicals, except sulfonamides and boric acid, most of which are known to be responsible for so-called idiosyncracies or allergic diseases of man, were able to sensitize guinea pigs in several ways, that definite manifestation of anaphylaxis to such substances, excluding iodine which showed distinctly hypersensitive response in Dale test, seemed to be limited to reactions of the skin, and that horse serum or swine serum added to chemicals tended to accelerate their anaphylactogenic ability. It was also revealed that hypersensitivities to these substances were transferred by injec-
ting sera, euglobulins or pseudoglobulins. This fact proves, it would seem, that anaphylactic antibodies are contained in globulin fractions, and indicates that such allergic phenomenon induced by simple chemical compounds were caused by the reaction between an antigen and its antibody, as in anaphylaxis of complex proteins. It must be added that some attempts were made in this laboratory to prove the presence of antibodies to chemicals in the serum by test tube experiments, but no positive result have been obtained.

**Duration and Intensity in Relation to Sensitizing Procedure**

To elucidate the effect of difference in sensitizing methods upon the incubation period, duration of hypersensitivity and intensity of allergic reactions, T. Yamasaki has carried out a series of experiments with Lugol's solution and formaldehyde. Lugol's solution was used to sensitize guinea pigs in four ways, i.e., 1) the same amount of sensitinogen was given by the same number of injection through different routes, 2) the same amount was given through the same route by different number of injection, 3) different amounts were given by the same number of injection through the same route, or 4) different quantities of sensitizing serum were injected through the same route by the same number of injection (passive sensitization). Procedures and findings of his experiments were as follows: Lugol's solution containing 10% of horse serum was always used in sensitization. Intestinal reaction to the addition of 1.0 cc of 1:100 Lugol's solution was detected, after the last sensitizing injection, on the 4th day and at the beginnings of the 1st, 2nd, 4th, 6th, 8th and 10th weeks. In case of passive sensitization, it was tested 6 hours, 12 hours, 1 day, 3 days, 5 days, 7 days and 9 days after the injection of the serum. In the first experiments, a single dose was injected into each of three groups of guinea pigs; to group A, 0.25 cc of 1:3 Lugol's solution was given intracutaneously, and to group B, 0.5 cc of 1:6 solution was administered intraperitoneally, while group C received intravenous injection of 0.5 cc of 1:6 solution. In each group, hypersensitivity began to present itself on the 4th day, and apparently three groups did not show a difference in the incubation period. But, from the second week an obvious difference in the intensity was seen, group A displaying stronger responses than B or C groups. Duration of hypersensitivity was longer in group A and shorter in group C than in group B; even in the 10th week there were some cases of the positive reaction in group A, whereas hypersensitivity of group C subsided by the 6th week. In the second experiments, 10 daily intracutaneous injections of 0.05 cc of 1:6 Lugol's solution were given to group D, and the result was compared with that of group A. In group D, marked allergic reactions appeared in the first week, but no positive reaction took place in the 10th week, which indicated shorter incubation and duration than in group A. In the third experiments, 10 daily intracutaneous injection of 0.1 cc of 1:6 Lugol's solution were administered to group E. There seemed to be no difference in incubation, intensity or duration between this group and group D. In the fourth experiments, 1.0 cc of the serum of the guinea pig actively sensitized with Lugol's solution was intravenously given to group F, 2.0 cc to group G. Comparison of the two groups disclosed a
tendency that the reaction was more intense and the duration was longer in Group F. In parallel with this, experiments with egg albumin were carried out by the same procedures, and similar results to those of iodine anaphylaxis were obtained.

Furthermore, he has made observations in like manner on the allergic skin reaction induced by formaldehyde. In sensitization, 1:30 or 1:300 formalin mixed with horse serum at the ratio of 9:1 was used and the skin reaction was tested with 0.1 cc of 1:800 pure formalin solution. Single sensitizing injections were given to three groups as follows: an intracutaneous dose of 0.1 cc of 1:30 formalin solution to group A, an intraperitoneal dose of 1.0 cc of 1:300 solution to group B, and an intravenous dose of 1.0 cc of 1:300 solution to group C. And, to group D 10 daily intracutaneous injections of 0.1 cc of 1:300 solution were applied. The skin reaction began to appear on the 4th day and the allergic state continued till the 10th week, alike in groups A, B and C. Intensity of the reaction was seen to be slightly stronger in group A than in B and C groups. In groups D, a vivid reaction was incited on the 4th day and most of the animal displayed marked reaction from the first week, hypersensitivity not disappearing even in the 10th week.

Recently, other workers in this laboratory have undertaken a study on the same subject, utilizing the finding by T. Sasaki and Y. Kobayashi that anaphylaxis to simple chemical compounds could be induced in rabbits as well. In their experiments, the highest dilution of potassium bichromate that instigated the skin reaction was used as the titer of hypersensitivity of the rabbit, in contrast to Yamasaki who measured hypersensitivity by intensity of reactions, cutaneous and intestinal, caused by borderline amounts of a provocative agent. For sensitizing purpose, they gave the same total amount of the chemical to each group of 3 to 7 rabbits in the following ways: a) 0.05 cc of 1% solution was injected daily for 10 days through the intracutaneous route; b) 0.2 cc of 0.25% solution was injected daily for 10 days through the intracutaneous route; c) a daily dose of 0.2 cc of 0.5% solution was intracutaneously injected for 5 days; d) 0.2 cc of 0.5% solution was given daily for 5 days through the oral route; e) a daily dose of 0.2 cc of 0.5% solution was injected for 5 days through the intravenous route; and f) 0.2 cc of 0.5% solution was injected daily for 5 days. Skin reactions were examined with 0.1 cc of the bichromate dilutions from 1:3,000 up to 1:50,000. And, starting from the 7th day after the last sensitizing injection, this test was tried weekly until hypersensitivity disappeared. Most of the animals displayed titers 4,000 to 10,000 in the first week of the test, and their highest titers of 20,000 to 50,000 were reached during the third to the 5th week, but in the 12th or 13th week their titers fell to around 3,000 to 7,000 and tapered down thereafter, although there was an exceptional one which showed the titer of 4,000 in the 19th week, after exhibiting unusually high titers throughout the test period. Taking as a whole, a), b) and c) groups gave the best results in titers and duration of hypersensitivity and e) and f) groups brought out the poorest. These findings of Yamasaki and others substantiated that anaphylaxis to chemicals are induced through various routes and that the most efficacious method is to sensitize intracutaneously by several daily injections, and exposed also that hypersensitivity to chemicals remains for a fairly long period of time.
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