

# Vaccines containing organic mercury compound and implications in relation to BSE (bovine spongiform encephalopathy)

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## ABSTRACT

The present study was undertaken to investigate the effect of thimerosal (an organic mercury compound), a preservative incorporated into vaccines, on living organisms. A case is presented where BSE-like pathological conditions in brain tissue is artificially created. Possible mechanisms and causes are discussed. Implications are commented on. In this study our experiments revealed production of similar BSE-like pathological conditions through in vivo staining of a chemically affected tissue, from which we inferred that BSE is a disease caused by chemical contamination.

**Key words:** BSE, (bovine spongiform encephalopathy), organic mercury compound, thimerosal

## Introduction

According to current information on BSE, onset of BSE is considered to be caused by biological factors such as viral infection and abnormal multiplication of pathogenic protein, however no disease-creating mechanism has yet been specified (Ozawa, 2001a,b, 2002, 2003a,b, 2004). In this study our experiments revealed production of similar BSE-like pathological conditions through *in vivo* staining of a chemically affected tissue, from which we inferred that BSE is a disease caused by chemical contamination.

Specifically, BSE-like conditions were produced by immersing a brain tissue sample in a vaccine product containing thimerosal and aluminum, and allowing the ingredients to infiltrate the sample from the tissue surface. We adopted this approach with an aim to approximate the conditions of the brain after it received a thimerosal containing agent, since mercury introduced in biological tissue is known to be accumulated in the brain after being taken up by capillarity through lymph ducts and passing through the spinal canal. The thus-created pathological conditions were visually observed under a microscope.

The disease-creating mechanism is summarized as follows: Organic mercury and aluminum are brought into contact in a biological organism, in particular brain tissue, whereby amalgamation of the two substances occurs *in situ*. At this time, heat of reaction burns the brain tissue and creates pathological lesions, which we have confirmed are consistent with the reported profile of BSE. The experimental data also highlighted that BSE (bovine spongiform encephalopathy), Minamata disease, and Creutzfeldt-Jacob disease share some common symptoms.

We also noted that specific chemical substances; i.e., an organic mercury compound and an aluminum compound, participate in both formation of BSE-like pathological conditions and generation of a variant strain of a bird flu virus. Thus, we concluded that the pathological conditions might be caused by inoculation of a preventive vaccine containing an organic mercury compound and an aluminum compound, or by a disinfectant containing the same compounds.

## Material and methods

### *Experiment 1*

Quantification of vaccine additives and changes in measurements caused by amalgamation

1-1 Reaction of additives contained in vaccine

#### *Objective*

“Standards for Veterinary Biological Products” (spectrophotometry; UV method) is the quantification method established under relevant law in Japan for assaying thimerosal and aluminum additive content in vaccines.

Quantitatively assay both thimerosal and aluminum additives in samples using “Standards for Veterinary Biological Products” (quantification method; established under relevant law in Japan) and through atomic absorption photometry.

#### *Vaccines employed*

Sample 1: Bivalent bovine salmonella vaccine (product of Kitasato Institute)

Sample 2: Salmonella Dublin-Typhimurium Bacterin Serial 172. final bulk sample, date mixed: 6-01-01

#### *Method*

Thimerosal and aluminum were quantitatively assayed according to "Standards for Veterinary Biological Products" (spectrophotometry; UV method; quantification method;

established under the relevant law in Japan) and through atomic absorption photometry.

*Results*

The results are shown in Table 1. As shown in Table 1, in the quantification of both thimerosal and aluminum, different measurement methods produced different quantification results. Aluminum was not detected by the method of "Standards for Veterinary Biological Products." This is presumably because the quantification method described in this protocol is primarily for analyses of aluminum ions, and therefore, the method could not detect aluminum oxide (Al<sub>2</sub>O<sub>3</sub>), which is considered to have been produced as a result of a reaction between mercury (Hg) contained in thimerosal—an ingredient of the sample—and added aluminum hydroxide (Al<sub>2</sub>(OH)<sub>3</sub>). Formation of aluminum hydroxide is supported by the following: 1) Atomic absorption photometry, which is a more reliable analytical method for the conditions of the present experiment, detected the presence of aluminum, and 2) in both samples, small amounts of sediment (Al<sub>2</sub>(OH)<sub>3</sub>) were observed.

The reaction between mercury and aluminum is considered to be an amalgamation process, in which aluminum is likely to be retaken into the thimerosal structure, in turn raising the mercury level commensurate with the amount of aluminum, as confirmed by spectrophotometry, which is the basis for "Standards for Veterinary Biological Products (quantification method)." Note that spectrophotometry cannot differentiate aluminum from mercury. As shown in Table 1, the amount of thimerosal assayed according to the method described in "Standards for Veterinary Biological Products (quantification method)" is approximately the same as the value calculated by summation of total Hg and aluminum as measured through atomic absorption photometry.

Table 1 Quantification results of samples

	Thimerosal		Aluminum	
	Sample 1	Sample 2	Sample 1	Sample 2
Standards for Veterinary Biological Products	0.0087 w/v%	0.0073 w/v%	Not detected	Not detected
Atomic absorption photometry	0.0039 w/v%	0.0045 w/v%	0.0018 w/v%	0.0025 w/v%

Note) The thimerosal levels identified through atomic absorption photometry denote values calculated on the basis of total Hg in the samples.

Sample 1

$$\frac{0.0087}{(i)} \quad 0.0039 + 0.0018 = \frac{0.0057}{(ii)}$$

(Hg)      (Al)

Sample 2

$$\frac{0.0073}{(i)} \quad 0.0025 + 0.0045 = \frac{0.0070}{(ii)}$$

(Al)      (Hg)

Unit: w/v%

(i): Quantitative values obtained according to "Standards for Veterinary Biological Products (spectrophotocolorimetry; UV method)"

(ii): Total values of quantitative data of Hg and Al obtained from atomic absorption photometry (AA method)

As shown above, in each sample, measurements (i) and (ii) were found to approximate to each other.

Note) In the manufacture of a thimerosal-containing drug product, thimerosal content of the product is controlled to 0.01% as determined by the assay method described in "Standards for Veterinary Biological Products."

### **Experiment 1-2** Reaction of thimerosal-containing vaccine and aluminum

#### *Objective:*

To determine if a heat generating amalgamation reaction would occur if thimerosal-containing vaccine is placed in contact with aluminum.

*Vaccine and material employed:* Influenza vaccine, Aluminum foil

#### *Method:*

Several drops of influenza vaccine were placed on aluminum foil, and tightly wrapped therein so as not permit leakage. For about one minute, pressure was applied with the fingers to eliminate the inside air, after which the aluminum foil with the vaccine was allowed to stand at room temperature. Forty-eight hours later, the aluminum foil was opened and the inside surface of the foil was observed with the naked eye and under a microscope.

#### *Result:*

Observation under a microscopic identified a countless number of pits on the inner surface of the foil as shown in Fig. 1. White crystals were found to be present along the peripheries of the pits. Microscopic observation of an area including pits revealed that the peripheral areas of the pits exhibit gloss and roundness, indicating that the pits are formed not through corrosion, but rather through melting of the metal (Fig. 2). In this experiment, when the vaccine was wrapped in aluminum foil and finger-pressed for about one minute, a considerable amount of heat was generated. The heat generation is presumably caused by the amalgamation process between Hg contained in thimerosal, which is a vaccine ingredient, and aluminum. Also, the crystals surrounding the pits are considered to be aluminum oxide produced through the heat-generating process.

### **Experiment 2**

Denaturation of protein by additives contained in vaccine

#### *Experiment 2.1* Protein coagulation by thimerosal

##### *Objective*

If protein and thimerosal are combined what reaction might occur and whether such a reaction alter the protein.

##### *Materials*

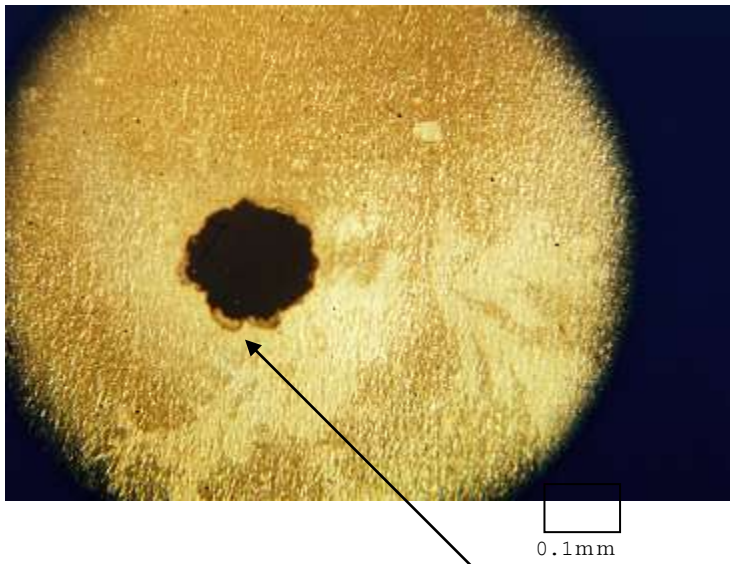
Egg white (raw material of influenza vaccine), Thimerosal (1 w/v% aqueous solution), and Physiological saline.

##### *Method*

Physiological saline was added to egg white from one egg, making a total volume of 500 mL (the volume will be called the "specimen" in this section). Thimerosal was added thereto in an amount of 0.01%, and the mixture was stirred with a magnetic stirrer for 30 minutes. Subsequently, centrifugation was performed at 3,000 rpm for 60 minutes. The supernatant was taken up and used as a measurement sample (Sample A).

Figs. (Photographs)

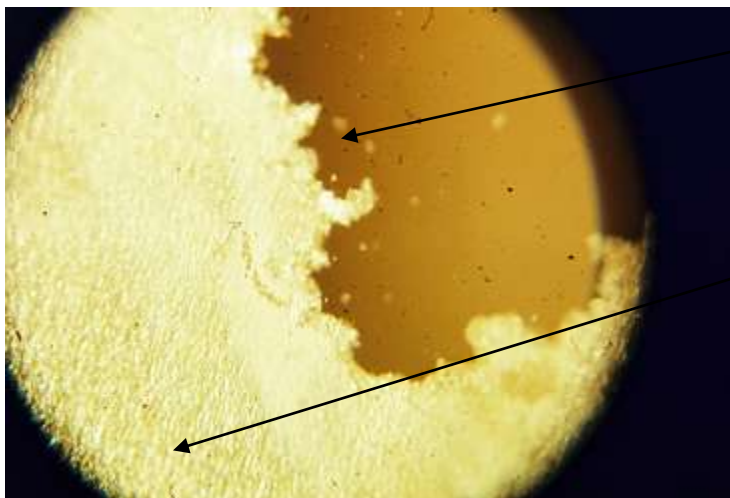
Fig. 1



Several drops of influenza vaccine were added onto aluminum foil. The foil was tightly folded in two under pressure and allowed to stand for 48 hours, during which pits were formed. (× 100)

Somewhat rounded peripheral portions indicate that the aluminum foil was melted.

Fig. 2



Melted

The foil surface is melted to become thin, creating wrinkles.

× 100

As a control, a comparative sample; i.e., Sample B, was prepared by combining a 10 mL aliquot of the specimen (which is an identical one as described above but separately prepared), and 90 mL of physiological saline (total volume: 100 mL), and collecting a supernatant in the same manner as employed for the preparation of Sample A.

An aliquot of 1 mL was taken from each of the Samples A and B, and subjected to a test for determining COD (chemical oxygen demand) and also to atomic absorption photometry for determining total Hg. Simultaneously, molecular weight distribution was determined through size exclusion high performance liquid chromatography (SEC) employing a TSK gel G3000SWxl column, and the protein level was investigated through infrared absorption spectrometry.

### Results

The results are shown in Tables 2 and 3. As compared with the comparative sample (Sample B), Sample A—which was a supernatant obtained by adding thimerosal and removing the precipitating sediment—showed a 50% reduction in COD. This reduction is presumably caused by the mechanism in which organic substances (COD components) undergo a solid-forming reaction and are removed in the form of sediment, reducing the concentration of amino acids or other protein components. Moreover, since the added thimerosal was also consumed by protein, residual Hg was lowered to 40% with respect to the entire amount of the added Hg. The above results suggest that, when bound to protein, thimerosal becomes insoluble and precipitates as a sediment. In fact, Sample A produced a small amount of white solids, which are considered to have been produced from such a coagulation reaction.

After the samples underwent the mentioned treatment, the molecular distribution of the remaining protein in the measurement sample (i.e., Sample A, thimerosal added) was found to exhibit an increase, of about 10%, in low-molecular weight amino acids as compared with the control sample (i.e., Sample B, without addition of thimerosal). After consideration of possible influencing factors attributable to the test method, the treatment is still believed to have changed the distribution of constitutional amino acids.

Table 2 COD (chemical oxygen demand) and detected Hg

	COD	Hg
Test sample (Sample A)	3,650 mg/kg	60 mg/kg
Control (Sample B)	7,160 mg/kg	not detected

Note: Since the control sample is a 10-fold dilution, the figures indicated for the control are those calculated accordingly.

Table 3 Protein distribution

Molecular weight distribution	% Peak area	
	Sample A	Control (Sample B)
≥ 700,000	Trace amount	1
300,000 - 700,000	0	0
100,000 - 300,000	1	1
30,000 - 100,000	87	96
10,000 - 30,000	Trace amount	Trace amount
≤10,000	12	2
Total	100	100

Analyzed by Japan Food Research Laboratories

## Experiment 2.2 Human serum agglutination induced by thimerosal-containing vaccine

### Objective

When a vaccine containing a thimerosal additive is injected into the body by inoculation it first comes in contact with blood. The objective was to determine what effects a vaccine containing a thimerosal additive might have on human blood.

### Vaccine and Materials

Fresh human blood (blood type B, Rh+), male Anti-A serum: Bioclone (product code: BAA201A21), Anti-B serum: Bioclone (product code: BBB592A21), Japanese encephalitis vaccine (Kitasato Institute, product code: 122-2)

### Method

Freshly drawn blood was employed. To the blood, an inactivated vaccine product (Japanese encephalitis vaccine) was added (a test sample). Separately, comparative samples were prepared by adding an anti-serum which is employed for determining a blood type of the ABO blood system. Since the materials other than human blood are all commercial products, we assumed that the test system was not affected by changes in pH or osmotic pressure. Accordingly, the samples were prepared by mixing fresh blood and the vaccine product or mixing fresh blood and either one of the anti-serum product (no other additives were added).

Table 4 Samples prepared

Sample No.	Constitution	Agglutination
1	Fresh blood alone	-
2	Fresh blood + anti-A serum	-
3	Fresh blood + anti-B serum	+
4	Fresh blood + Japanese encephalitis vaccine	+
5	Fresh blood + anti-A serum + Japanese encephalitis vaccine	+

### Results

Photomicrographs of the respective samples are shown in Figs. 3 to 8. Although observation with the naked eye did not detect any agglutination in sample No. 4, when observed under a microscope at a magnification of  $\times 100$  or more, blood cells were clearly seen to be agglutinated (Fig. 6). Deformation of the outer membrane of the blood cells was also confirmed. Sample Nos. 4 and 5, which were considered not to agglutinate, exhibited agglutination (see Figs. 6 and 7). Vaccine-added samples showed deformed outer surfaces of red blood cells, and apparently, only the outer membrane was shrunken and produced wrinkles. During the course of application of the samples onto slide glasses and drying, thimerosal crystals were deposited around the red blood cells which served as the nuclei for crystal growth (Fig. 8). Since the vaccine-containing samples were prepared by simply mixing fresh blood and a vaccine product for human use and no special manipulation had been effected, deformation in blood cell walls was suggested to be attributed to inherent properties of the vaccine or the solvent used for the vaccine.

Figs. 3 to 8 (Photographs)

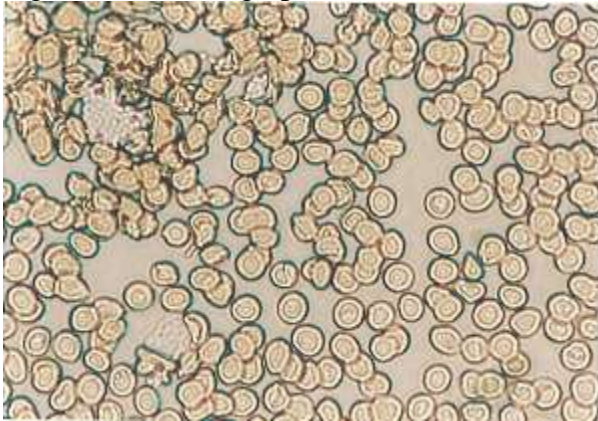


Fig. 3 Control

Healthy blood cells  
(not treated) (Rh+B)

×150



Fig. 4 Fresh blood +  
Anti-A serum

The blood does not  
agglutinate upon  
addition of ABO blood-  
typing anti-A serum

×100

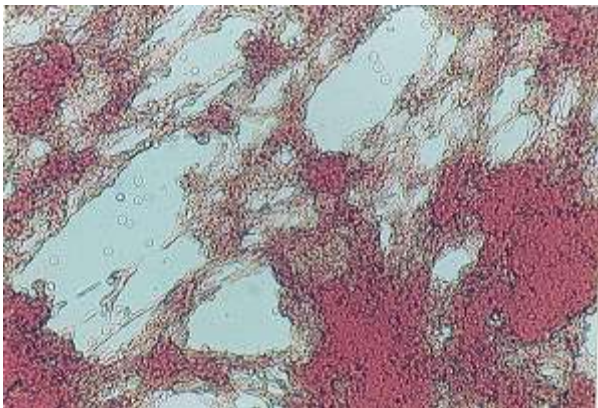


Fig. 5 Fresh blood  
+ Anti-B serum

Agglutination upon  
addition of ABO  
blood-typing anti-B  
serum

×100





Fig. 6 Fresh blood + Japanese encephalitis vaccine

Immediately after addition of Japanese encephalitis vaccine at a ratio of 1 (blood) : 0.5 (vaccine). Observed after Japanese encephalitis vaccine was added to type B blood.

×100

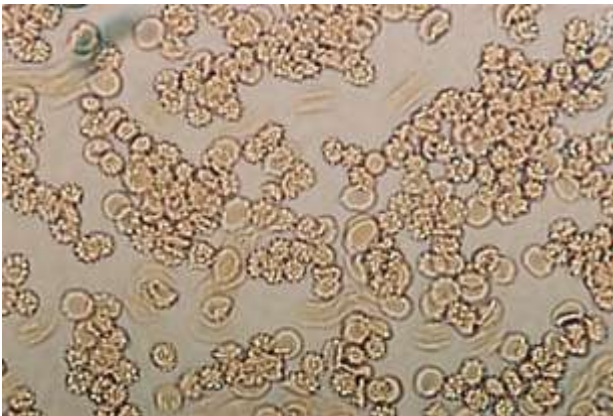


Fig. 7 Fresh blood + Japanese encephalitis vaccine + anti-A serum

Blood cells are agglutinated.

×150

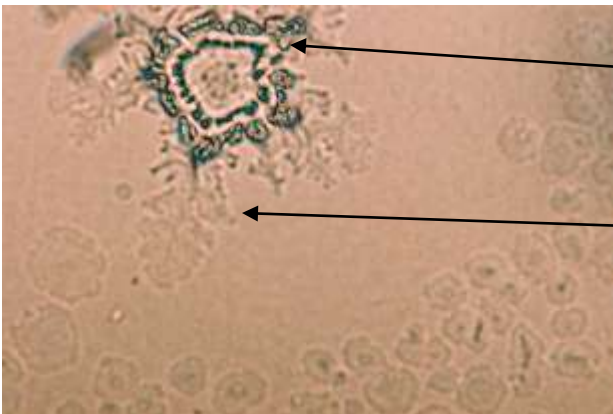


Fig. 8

- Outer membranes of the blood cells are deformed, and thimerosal crystals are deposited around the cells.
- Agglutinated blood cells have irregular shapes, and a crystal-like substance is observed at apical portions thereof.

×400

### **Experiment 2.3** Foreseeable clinical conditions resulting from possible biological reaction of thimerosal in the living body

#### *Objective*

What are the effects or changes to brain tissue when thimerosal contained in vaccines is absorbed and eventually reaches the brain?

#### *Vaccine and Materials*

Rat brain, Influenza vaccine, Rabies vaccine, Bovine Salmonella vaccine. Each vaccines each contain thimerosal and aluminum hydroxide.

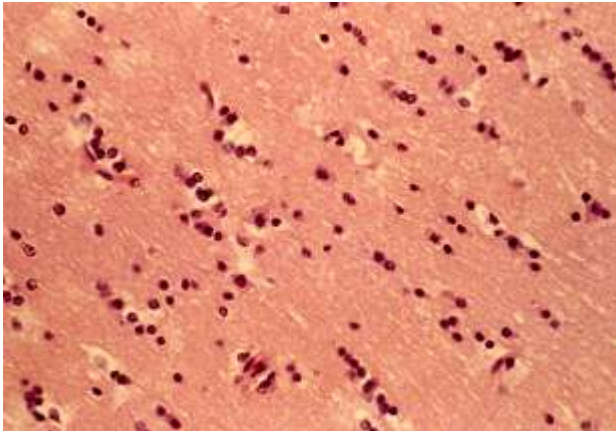
#### *Method*

Rats were sacrificed through excessive etherization, and immediately thereafter, they were exsanguinated so as to eliminate any possible effects resulting from the metabolic function of the animals. The brain was removed from each animal, and samples were prepared by immersing the brain in respective vaccines for 48 hours as indicated in Table 5. Subsequently, each vaccine-immersed brain was fixed with 10% formalin, then subjected to hematoxylin staining or immersion in 0.1% sodium thiosulfate, whereby pathological specimens were prepared for examination under a microscope. For each species of the above-listed vaccines; i.e., influenza vaccine, rabies vaccine, and bovine salmonella vaccine, specimens 1 to 3 were prepared.

Table 5 Specimen sources

Specimen 1	The brain was removed immediately after the animal was sacrificed, and stored in a 10°C refrigerator so as not to be frozen (Control specimen).
Specimen 2	Immediately after the rat was sacrificed, the skull was filled with influenza vaccine, and stored in a 10°C refrigerator so as not to be frozen. (After addition of vaccine, the skull was immersed in sodium thiosulfate solution.)
Specimen 3	The totally excised brain was divided into three parts, and each part was placed in a 3-mL glass vial. Influenza vaccine, rabies vaccine, and bovine salmonella vaccine were individually and separately added to the respective vials so that the brain parts were entirely submerged. Thereafter, the glass vials were stored in a 10°C refrigerator so as not to become frozen (Specimens for hematoxylin staining).

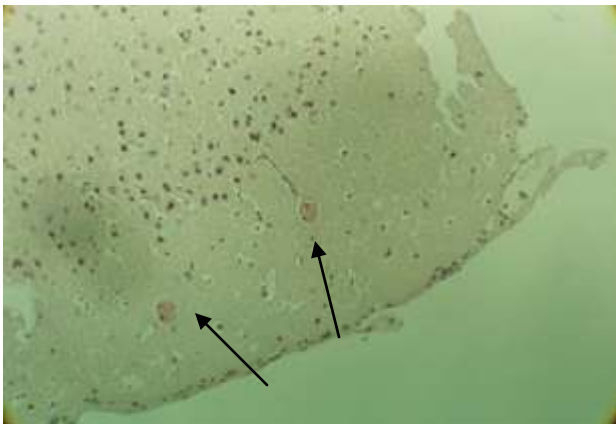
Results



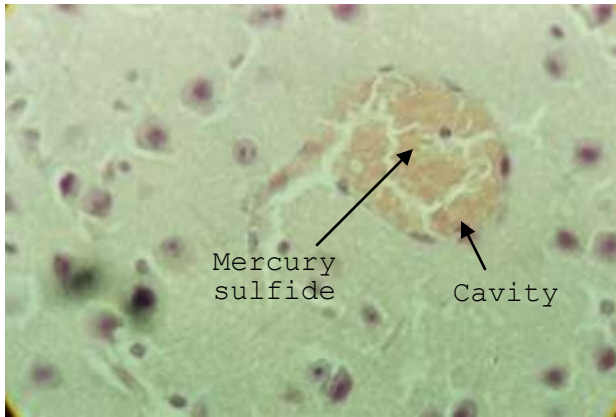
Typical bovine brain tissue after onset of BSE (news photograph)



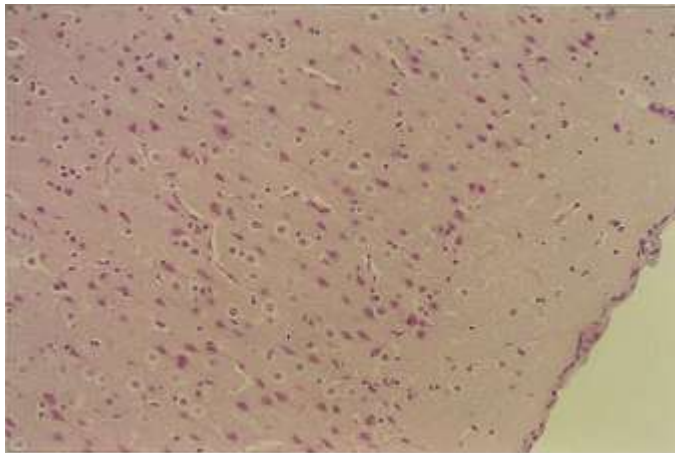
Rat brain tissue which was treated with vaccine then immersed in sodium thiosulfate. The brown portions are considered to be a sulfide generated as a result of reaction between mercury that was collected in cavities formed through burning and sodium thiosulfate (mercury sulfide compound: brown color) No. 1  
×100



Rat brain tissue which was treated with vaccine then immersed in sodium thiosulfate. The brown portions are considered to be a sulfide generated as a result of reaction between mercury that was collected in cavities formed through burning and sodium thiosulfate (mercury sulfide compound: brown color) No. 2  
×100

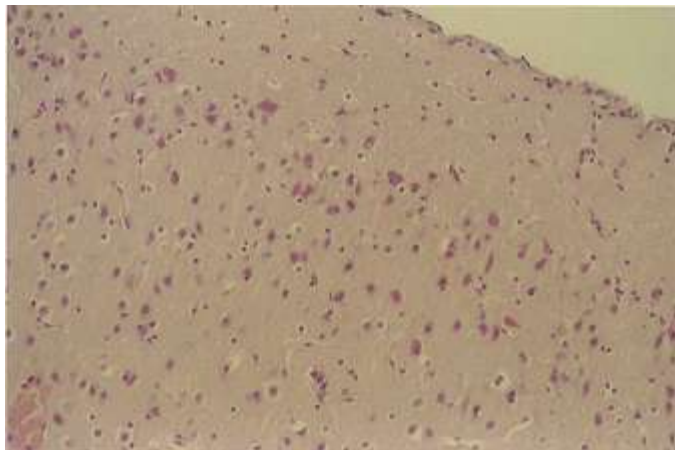


Rat brain tissue which was treated with vaccine then immersed in sodium thiosulfate. The brown portions are considered to be a sulfide generated as a result of reaction between mercury that was collected in cavities formed through burning and sodium thiosulfate (mercury sulfide compound: brown color) No. 3  
x400



Nerve cells of rat cerebral neocortex (control), untreated, hematoxylin staining

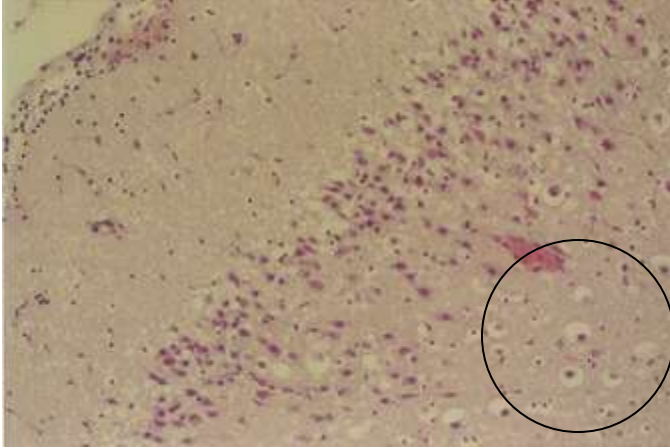
x100



Nerve cells of rat cerebral neocortex (treated with bovine salmonella vaccine), hematoxylin staining

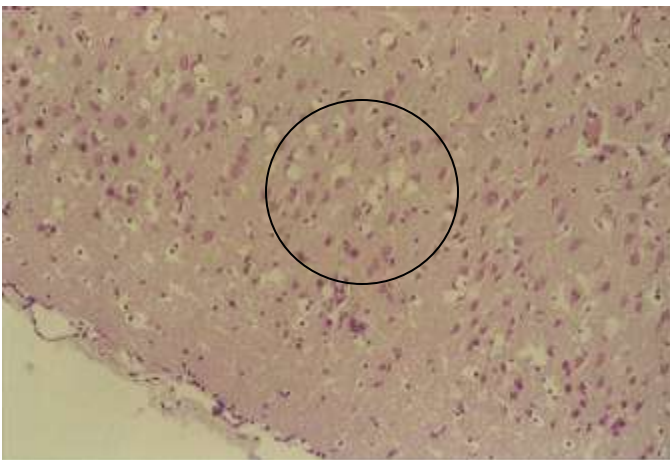
x100





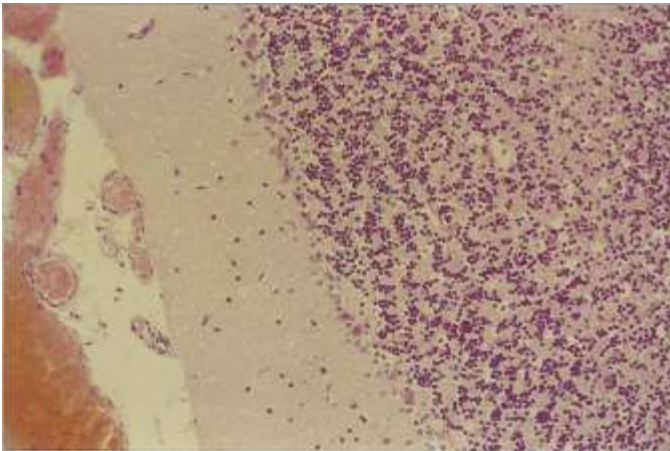
Nerve cells of the surface layer of rat cerebral neocortex (treated with rabies vaccine). Cavities are observed. Hematoxylin staining

x100



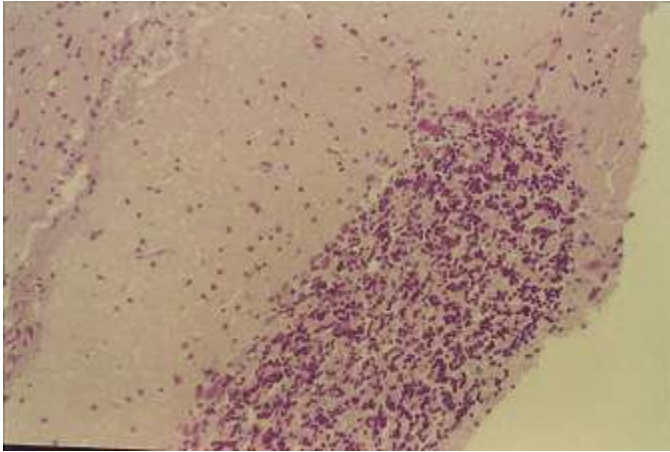
Nerve cells of rat cerebral neocortex (treated with bovine salmonella vaccine). Cavities are observed. Hematoxylin staining

x100



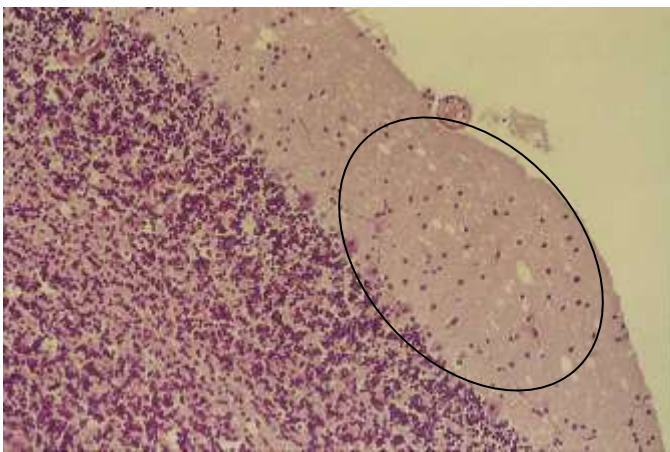
Rat cerebellum Purkinje cells (control), untreated, hematoxylin staining

x100



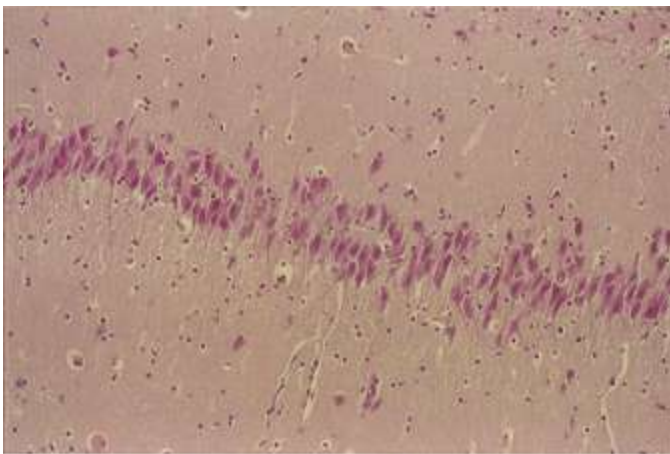
Rat cerebellum Purkinje cells (treated with bovine salmonella vaccine). Cavities are observed. Hematoxylin staining

x100



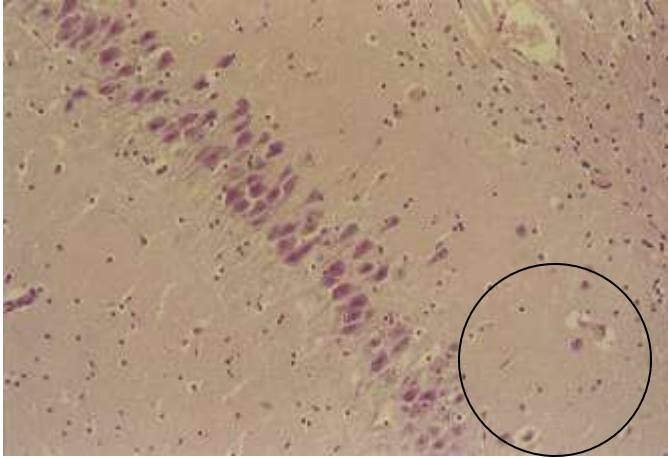
Rat cerebellum Purkinje cells (treated with rabies vaccine). Cavities are observed. Hematoxylin staining

x100



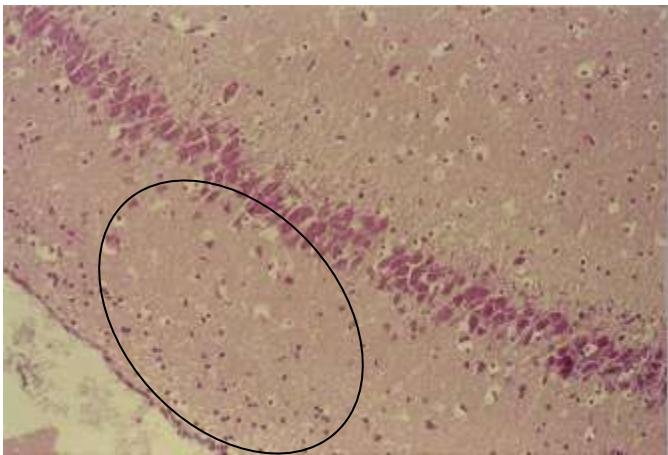
Rat hippocampus (dentate gyrus) nerve cells (control), untreated, hematoxylin staining

x100



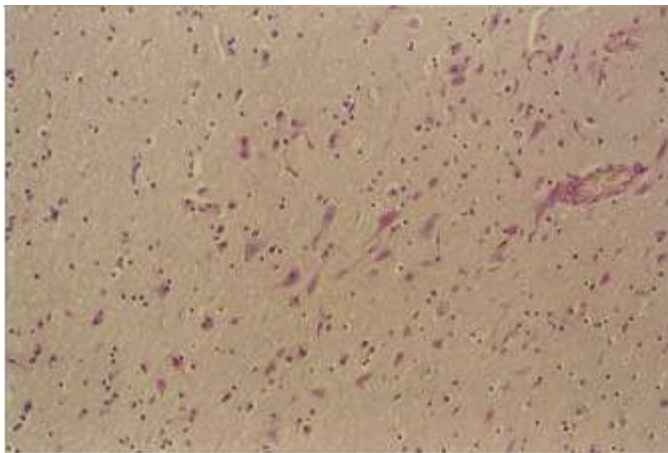
Rat hippocampus (dentate gyrus) nerve cells (treated with bovine salmonella vaccine). Cavities are observed. Hematoxylin staining

x100



Rat hippocampus (dentate gyrus) nerve cells (treated with bovine salmonella vaccine). Cavities are observed. Hematoxylin staining

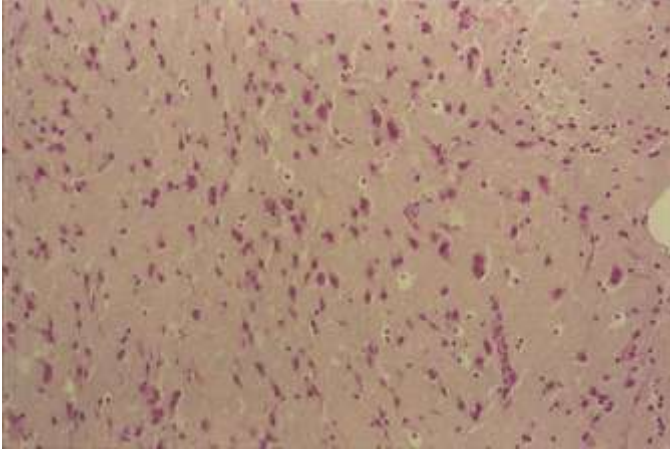
x100



Rat basal ganglia nerve cells (control), untreated, hematoxylin staining

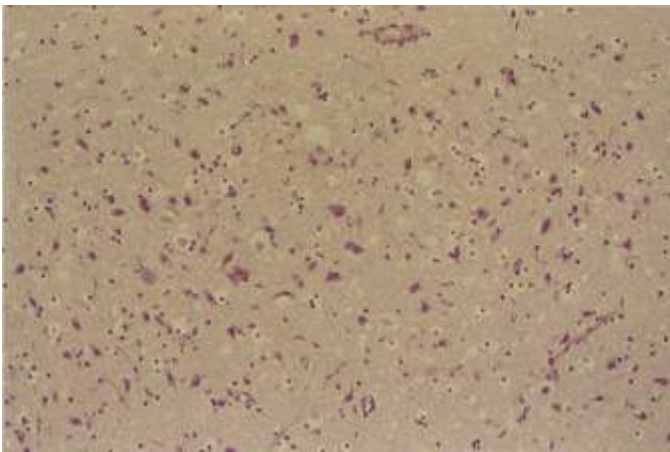
x100





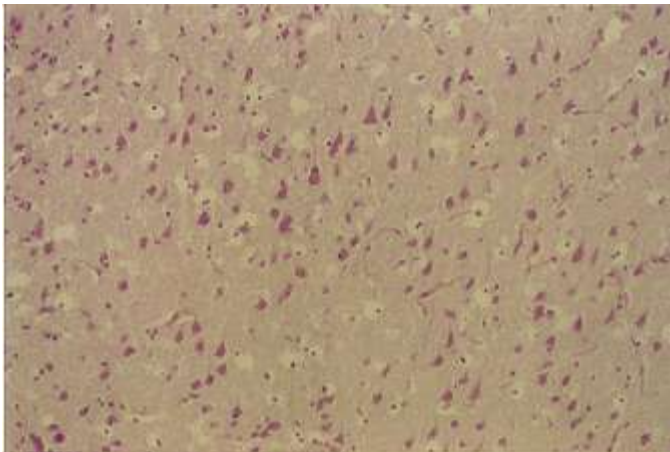
Rat basal ganglia nerve cells (treated with bovine salmonella vaccine). Cavities are observed. Hematoxylin staining

x100



Rat basal ganglia nerve cells (treated with bovine salmonella vaccine). Cavities are observed. Hematoxylin staining

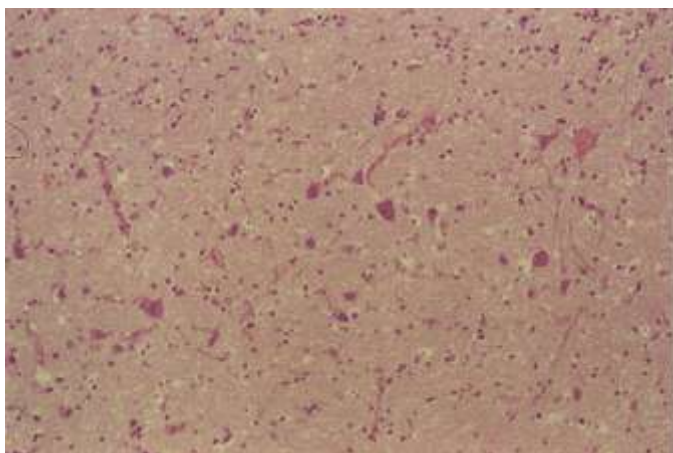
x100



Nerve cells of rat brain, deep in the cerebrum (treated with rabies vaccine). Cavities are observed. Hematoxylin staining

x100





Rat midbrain nuclei  
(treated with influenza  
vaccine). Cavities are  
observed. Hematoxylin  
staining

×100

### Experiment 3

Hg level in meat-and-bone meal

#### *Objective*

To investigate the reason why meat-and-bone meal for feed is not putrefied

#### *Material*

Meat-and-bone meal for feed (produced in Iwate Prefecture around 2002)

#### *Method*

JIS K 0102 66.1.1, Reduced vapor atomic absorption spectrometry

#### *Results of measurement*

Total Hg: 0.0630 mg/kg

#### *Discussion*

The employed meat-and-bone meal sample was placed in an ordinary polyethylene bag immediately after purchase and stored for 6 months at ambient temperature. Since the meal did not show any sign of putrefaction, inclusion of some sort of preservative was suspected. As a possible preservative, organic mercury compounds were chosen as a target substance for investigation. The detected Hg level was slightly in excess of 12 times the threshold level (0.005 mg/kg) permitted for, for example, soil according to the environmental quality standards, thus clearly indicating hazardousness.

In the manufacture of the meat-and-bone meal, preservatives are not added. Therefore, the reason why such a high level of Hg was detected remains unknown. However, a possible explanation is that preventive vaccine inoculation served as the source of Hg, which accumulated in bones, etc., which are the raw materials of the meal.

### SUMMARY AND REVIEW

1. When a vaccine product containing thimerosal and an aluminum compound is brought into contact with aluminum foil, amalgamation occurs, resulting in melting of the foil. In the manufacture of a vaccine product, at the time of addition of thimerosal, aluminum is amalgamated with Hg present in the molecular structure of thimerosal. This reaction was found to be such a violent exothermic reaction (oxidation reaction) that brings about melting of the aluminum foil. Incorporation of thimerosal (serving as a preservative) and aluminum hydroxide (serving as an adjuvant) into a single product is considered problematic in that it may possibly impair the function of aluminum hydroxide as an adjuvant and it causes heat generation, leading

to denaturation of major protein components of a biological product containing these substances. Accordingly, the combined use of thimerosal and aluminum hydroxide in a vaccine product should be avoided or some countermeasures must be taken.

2. Sedimentation was observed when thimerosal was added to egg white diluted with physiological saline. The COD value as measured in supernatant was about 1/2 that of the control. After treatment, the amino acid fraction of MW 100,000 to 300,000 decreased and that of MW  $\leq$  10,000 increased, as compared with the control.

From this, thimerosal, which is added to vaccines during manufacture thereof, was considered to cause partial denaturation of protein (i.e., antigen) of the strain of a disease prevention target during the manufacture of vaccines. This means that a recipient of the vaccine will come to receive an antigen that is different from that originally intended. Thus, the original aim of vaccination cannot be attained. Specifically, the amino acid sequence of an antigen receptor will be altered, and when the receptor has come to have such a morphology that does not fit the target strain, a new antibody will be created in the vaccine recipient, preventing attainment of the intended effect. According to this hypothesis, at every stage where thimerosal is added, a new, adventitious antigen will be produced.

3. When a vaccine product containing thimerosal and aluminum is brought into contact with blood, the cell walls of red blood cells are shrunken and deformed.

4. Organic mercury was detected in a meat-and-bone meal feed. We detected a high level of Hg in a meat-and-bone meal feed, which is an alleged cause of BSE. If the detected Hg originates from thimerosal, this explains why the meal is not putrefied after a prolonged period (even years) of storage under non-sterilized conditions, and explains Hg accumulation in the body through the food chain.

5. When a vaccine product containing thimerosal and aluminum is brought into contact with rat brain, BSE-like pathological changes were induced in the brain tissue. From this finding, we consider that there possibly exist similar relations, in terms of disease onset mechanism and organic mercury, between Minamata disease and organic mercury; mad cow disease and bovine salmonella vaccine; Creutzfeldt-Jacob disease and disinfection with merzonin during dura mater transplant; and emergence of a new variant strain of a bird flu virus and a Newcastle vaccine. Since the mentioned diseases exhibit similar clinical conditions, they are thought to be disorders caused by accumulation of vaccine-originating organic mercury in the brain tissue.

#### *Acknowledgement*

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## POSTSCRIPT

Minamata disease has been reported as a brain disorder which is caused by organic mercury accumulated in the body through the food chain. BSE is reported to be linked with inoculation with large amounts of bovine salmonella vaccine containing thimerosal and aluminum hydroxide to cattle over several years until shipment of the cattle. Creutzfeldt-Jacob disease is related to treatment with merzonin, a disinfectant, in dura mater transplant. Thus, an organic mercury compound is apparently involved in these diseases. Moreover, according to recent information, a new strain of chicken-derived bird flu virus has emerged. Since farm-raised chickens are regularly inoculated with a Newcastle vaccine, emergence of a bird flu virus mutant may be caused by inoculation with large amounts of the Newcastle vaccine. (Note that bovine salmonella vaccines and Newcastle vaccines contain organic mercury).

Unlike Minamata disease, BSE and Creutzfeldt-Jacob disease are considered infectious diseases, but hitherto, no causal agent has been identified. Also, from the microbiological standpoint, incubation periods for these infections are considered very long. From these facts, the BSE-like conditions are predicted to be attributable to damage to the brain caused by burning from a chemical substance containing organic mercury, rather than attributable to infectious disease. In this study, we believe we have sufficiently substantiated this prediction.

However, we wish to invite researchers to conduct further trials to confirm our test results. In addition, we wish to call researchers' attention to the safety issue of U.S. beef. In Japan, Great Britain, and other European countries, large amounts of bovine salmonella vaccine are used for preventive vaccination, and therefore, if our theory is correct, emergence of BSE would naturally be expected. In contrast, the U.S. government goes one step further by prohibiting use of vaccine products containing thimerosal and aluminum. This supports the fact that BSE incidence is very low in the U.S.A. We consider U.S. beef to be safer than Japanese beef. We suggest that American researchers conduct follow-up studies of our experiments and confirm the safety of the U.S. beef. In addition, Japanese people should abandon the myth that beef is safe so long as it is produced in Japan. Otherwise, not only Japanese citizens but also people in other countries will continue to face the threat of BSE. We thus conclude our study by placing an emphasis on the necessity of avoiding use of an organic mercury compound as a preservative and an aluminum compound as an adjuvant, as stipulated by the American government. Effective handling of BSE and prevention of artificially-induced emergence of a new strain will be attained only after those substances are eliminated from vaccine products.

Based on the results of the present experiments, we have developed an "ultraviolet-ray irradiation apparatus for sterilization of a liquid or sludgy substance" (patented in Japan and the U.S.A.) which is an application of a static mixer. The apparatus enables production of sterile products without use of any preservative or similar materials. This apparatus is expected to contribute to rational utilization of UV sterilization in many technical fields, including UV sterilization of colored or opaque fluids such as jam or cream.

Lastly, we sincerely hope that American researchers verify our experiments. We would be honored if our study results draw their attention.

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