# BRAIN-EATING BUGS
# The Vaccine Connection

***The discovery that vaccines are contaminated with disease-causing amoebas should warrant a complete reassessment of immunisation policies.***

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Television newsreels all over Australia in January 1996 reported on the deaths of two five-year-old children, one in Adelaide and one in Tasmania, from "brain-eating amoebas". Listening to these startling reports, I remembered that when I was writing my book, *Vaccination*, and studying medical papers dealing with the contamination by monkey viruses of the monkey kidney tissues used in the production of the polio vaccine, one of the articles mentioned *Acanthamoeba* as yet another contaminant of these tissue cultures, besides the well-known and well-publicised simian viruses SV1-SV40.

As a matter of curiosity, I looked up the paper written by Hull et al. (1958) in my files, and there it was, on page 35: "Recently an ameba was isolated from monkey kidney tissue cultures and was identified as belonging to the genus *Acanthamoeba*. It grew readily in tissue cultures... It appeared to have the ability to infect and kill monkeys and mice following intracerebral and intraspinal inoculation."

Within a short period of time I was able to locate dozens more medical papers dealing with the pathogenicity of these amoebas in animals and, even more importantly, also in humans. For let's not forget that millions of children all over the world had been injected or orally administered a number of viral vaccines, and the polio vaccine in particular, cultured on the monkey kidneys.

## CONTAMINATION OF BIOLOGICALS, INCLUDING VACCINES

Contamination of vaccines by animal micro-organisms has been plaguing vaccine production from the very start and has been implicated in a number of serious diseases, leukaemia, cancer, SSPE (subacute sclerosing pan encephalitis) and even AIDS being the most prominent examples.

As recently as 1993, a journal called *Vaccine* published an article which admitted that "Virus-contaminated cell cultures are a major problem in the bio-industry... Cell cultures can be permanently virus-infected, or can become infected, usually through the use of contaminated sera."

## WHAT ARE AMOEBAS?

Amoebas are one-cell organisms—protozoans. According to an excellent review by Ma et al. (1990), amoebic protozoans are classified in the phyllum Sarcomastigophora. They also belong to Rhizopoda, meaning as equipped by propulsive pseudopodia and/or by protoplasmic flow without production of pseudopodia. Acanthopodina, a suborder of the order of Amoebida, form two families: Vahlkampfiidae and Acanthoamoebidae with two genera, *Naegleria* and *Acanthamoeba* respectively. A number of species have been recognised belonging to either of these genera.

Depending on living conditions, *Naegleria* species form three life-stages: trophozoite, flagellate and cyst. In contrast to this, *Acanthamoeba* species form only two life-stages: trophozoite and cyst. Initially, in the absence of evidence to the contrary, amoebas were considered harmless.

The first mention of "Free-living amoebae as contaminants in monkey-kidney tissue cultures" is that by Jahnes, Fullmer and Li (1957). Jahnes et al. (1957) isolated two strains of apparently the same amoeba from monkey kidney tissue cultures. They looked like rounded bodies, similar in appearance to cells manifesting changes induced by certain simian (monkey) viruses. However, on closer examination, they proved to be amoebic cysts. The cysts varied in size, usually from 10 to 21 microns in diameter. In one experiment, the cysts were treated with 10% formalin, washed and inoculated into monkey kidney tissue culture tubes. The monkey kidney cells fagocytised the cysts. The tropho-

zoites showed to be sensitive to temperature. Under refrigeration down to four degrees Celsius they turned into cysts. The cysts were resistant even under -50 degrees Celsius for months and survived in the pH range 5.0-9.0. They were not affected by streptomycin and penicillin in tissue cultures.

## AMOEBAS RECOVERED FROM MONKEY KIDNEYS KILLED ANIMALS

A series of articles started appearing in *Science* (1958) and *Journal of Pathology* (1959), written by Culbertson and collaborators, which confirmed that amoebas caused brain disease and death within days in monkeys and mice. They first found and recognised amoebas accidentally in the lesions of animals that died from the inoculation of a tissue-culture fluid of trypsinised monkey kidney cells. It was thought this was caused by yet another unknown monkey virus, but it was later shown to be *Acanthamoeba.*

Following intracerebral and intraspinal inoculation, "extensive choriomeningitis and destructive encephalomyelitis occurred" and killed monkeys in four to seven days and mice in three to four days. Intravenous injections of the amoebas resulted in perivascular granulomatous lesions. Intranasal inoculation in mice resulted in fatal infection in about four days. These mice exhibited ulceration of the nasal mucous membrane with direct invasion of the adjacent base of the skull and involvement of the frontal lobes of the brain. There were amoebas in the lungs, and they caused severe pneumonic reaction. Pulmonary veins were invaded and there were numerous thrombi containing many amoebas. Haemorrhage was a common feature. Sections of the kidney showed amoebas present in the glomerular capillaries (Culbertson et al., *Science*, 1958), which further demonstrated that *Acanthamoebae* were indeed pathogenic for monkeys and mice.

**Amoebas were found in swimming pools in Czechoslovakia where 16 young victims died of acute purulent meningoencephalitis, and thermal pools in northern New Zealand.**

Amoebas showed the ability to migrate through the tissues. The size of the inoculum did not matter much: both small and large inoculums produced amoebic invasions. Small inoculums tended to cause death by pulmonary invasion, while large inoculums were followed by brain invasions. Intragastric inoculations were unsuccessful most probably because amoebic cysts were shown to be dissolved by bile.

Culbertson et al. (1958 and 1959) concluded that without question the *Acanthamoeba* is able to enter the body by its own power and may be able to do so in nature as well. However, the question of possible contamination and introduction of *Acanthamoebae* via the polio vaccines was not mentioned.

## ISOLATION OF AMOEBAS IN SOIL & AIR, AND THROATS & NOSES OF PEOPLE

Further research concentrated on isolation of amoebas from water and soil, and throats and noses of healthy people.

The attention of amoeba researchers would appear to have been diverted away from the monkey kidney cultures and the polio vaccine.

Indeed, amoebas do occur widely in nature, e.g., in lakes, pools and mud puddles. Amoebas collected with mud from these lakes grew out *in vitro* on the fourth day. When inoculated into the nose, two mice became ill and were sacrificed before death. Amoebas caused brain abscesses in these mice.

Amoebas were isolated from the air (Kingston and Warhurst, 1969) in the UK during an investigation into the possibility of isolating respiratory syncytial virus, and from the surfaces in cubicles in which infants with acute bronchiolitis were being nursed.

A more extensive research has been carried out because of the possibility that these amoebas may be associated with disease. The first amoebas were isolated at Booth Hall Children's Hospital in the cubicle occupied by a ten-week-old infant with an illness diagnosed as acute bronchiolitis. First, only syncytial respiratory virus was isolated and the child was discharged, but later an unidentified cytopathic effect was noticed in the tissue cultures inoculated from three air samples, and the causative agent was provisionally identified as the "Ryan virus". Later isolations were made from a floor swab and from a sample of air aspirated through the bedclothes in a cubicle occupied by another infant with respiratory syncytial virus infection. Amoebas were isolated from one of the samples taken in the laboratory and were identified as *Hartmanella castellanii.*

It had been Pereira et al. (1966) who originally isolated "Ryan 1 virus" in a post-mortem bronchial swab of a seven-months-old baby boy, Ryan. Six days before admission, baby Ryan developed a sore throat and ulcers in the mouth which later spread to the face. He was unwell, could not suck and developed loose stools. The day before admission he developed a cough and started to vomit. He was drowsy and dyspnoeic, made jerky movements and died soon after admission. The chest X-ray revealed patchy consolidation and dilatation of the right side of the heart. Necropsy showed some emphysema, petechiae and small areas of congestion and alveolar haemorrhage in the lungs, a fatty liver, prominent mesenteric nodes, and mucopus in the ears. *Escherichia coli* bacteria were cultured from the ears. Death was diagnosed as due to a respiratory infection associated with encephalitis and hepatitis. Vaccination status was not given, although considering the age of the baby he would have received up to three doses of the DPT and polio vaccines.

Ryan 2 and Ryan 3 'viruses' were cultured from a man of 29 years and a woman aged 30.

Ryan 4 was isolated from a ten-year-old girl who was ill with fever, cough, mild generalised lymphadenopathy and splenomegaly, and some vomiting for four or five days before admission. Her mother suffered from a similar illness. Vaccination status was not given.

Ryan 5 was isolated from a two-year-old girl suffering from fever and convulsions and some congestion of the throat.

Ryan 6 was cultured from a four-year-old child with fever and cough. She was diagnosed with bronchitis and has subsequently recovered. Her two-year-old sister suffered similar symptoms at the same time. Syncytial respiratory virus was also isolated from these children.

Armstrong and Pereira (1967) identified the Ryan virus as an amoeba: *Hartmanella castellanii.* They had no doubt that these amoebas came from the human respiratory tract.

According to Casemore (1969), tissue cultures in laboratories may be contaminated by amoebas living in the air.

Amoebas, including those pathogenic to animals and humans, were found in swimming pools (Cerva et al., 1969) in Czechoslovakia where 16 young victims died of acute purulent meningoencephalitis, and thermal pools in northern New Zealand (Mandal et al., 1970; Cursons and Brown, 1972; and others). Four

fatal cases of primary amoebic encephalomyelitis occurred soon after the victims had been swimming in these thermal pools. *Naegleria gruberi* was identified as the causative organism.

A great number of papers continued to be published in medical journals dealing with cases of amoebic encephalitis. A number of children and young adults developed brain disease and died after swimming in lakes or falling and scraping their faces. While the small wound healed, weeks or months later they developed convulsions and other neurological signs and died within days of what was usually diagnosed as a fulminant encephalitis. Sometimes posthumously, but often in autopsies performed straight away after deaths, amoebas were found proliferating in the brains of these unfortunate individuals.

## AMOEBA RESEARCH IN AUSTRALIA

A lot of useful amoeba research has been done in Australia. In 1965, Fowler and Carter of the Pathology Department of Adelaide Children's Hospital described three cases—a nine-year-old boy and two eight-year-old girls. All were considered by their parents to be healthy and suffering no previous illness. The first symptoms, lethargy and disinterest in their usual activities, appeared on the fourth day before death. All children became feverish, unwell and complained of severe headache, sore throat and blocked nose. They were given antibiotics for "upper respiratory tract infection". However, within another day, the children's condition deteriorated, they started severe vomiting, lost consciousness and were taken to the local hospital with a provisional diagnosis of meningitis. Despite intravenous glucose-saline fluid, chloramphenicol, penicillin and sulphadiazine, they fell into coma and died of cardio-respiratory failure on the way to the Adelaide Children's Hospital.

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Case 4 was a 28-year-old man suffering very much the same symptoms as the three children, except for a two-week history of a sore throat and headache.

Post-mortem in all cases revealed lung oedema and vascular engorgement and in three of the hearts. The right-sided chambers were flabby and dilated, and the myocardium contained small foci of necrosis and inflammatory cell infiltration. The brain was also moderately swollen, with meningeal veins collapsed, superficial capillaries over the vertex engorged, and a few petechial haemorrhages present. The olfactory bulbs were very reddened, soft, and adherent by a mass of sticky exudate to the adjacent frontal brain cortex. Microscopic examination of the meningeal exudate revealed small amoebas, some of them degenerate. Interestingly, cultures of the brain and meningeal exudates from all the cases yielded no bacteria, tubercle bacilli, torula or viruses.

It was concluded that the isolated amoebas belonged to the genus *Acanthamoeba* and that the infection occurred via the olfactory route.

Carter (1968) and Carter et al. (1981) described a further six cases (four children aged seven to ten years, and two adults aged 28 and 37 years) and one case (a two-and-a-half-year-old girl from Perth, Western Australia) of amoebic meningoencephalitis in Australia.

Many other Australian authors contributed in a substantive way to the knowledge of amoebas and human diseases caused by them. However, none of these researchers ever mentioned a possible link of the diseases to recent vaccination and especially to vaccine boosters.

## AMOEBA RESEARCH IN OTHER COUNTRIES

Butt (1966) described three cases of amoebic meningoencephalitis in the United States and coined the term "primary amebic meningoencephalitis". All victims did extensive swimming and diving in small land-locked lakes within a two-mile radius in central Florida. The pathological findings were very much the same as those reported in the Australian cases. It was concluded that the soil amoebas are stimulated to excyst to the infectious or trophozoite form by gram-negative bacilli which are present in the faecal stream, in decaying vegetation and in some small lakes during summer, as well as in drainage ditches, swamps and sewage outfalls.

The above authors also concluded that other cases may be missed and that, furthermore, the human amoebic disease may not always have a fatal outcome. Some of Culbertson's experimental animals were giving signs of recovery from amoebic infection.

Indeed, Seidel et al. (1982) reported on a successful treatment of primary amoebic meningoencephalitis. A nine-year-old girl complained of a mild headache, nausea, vomiting and increased lethargy. On the morning of admission she was unresponsive. Moving amoebas were found in her cerebrospinal fluid (CSF). The girl and her family had been swimming in Deep Creek hot springs in the San Bernardino National Forest on two occasions before the onset of the girl's disease.

This was the same area which was a source of fatal infection with *Naegleria fowleri* primary amoebic meningoencephalitis in 1971 (Hecht et al., 1972). This patient was given antibiotics, antifungal agent and sulphosoxazol. Twenty-four hours later, the girl started recovering; her condition stabilised 48 hours later and gradually improved during one month's hospital stay. Cell counts and results of chemical studies of the spinal fluid remained abnormal for several months, but amoebas were not found after three days of treatment. It is of interest that specific antibodies were demonstrated in the girl's serum at seven, 10 and 42 days after admission, and that her father had a low titre of specific antibody but did not become ill. The mother's serum was negative for antibody.

A chronic amoebic meningoencephalitis was described in a 30-year-old patient in Nigeria (Cleland et al., 1982). He had a five-year history of sleep disturbance that culminated in a confusional illness with convulsions from which he made a partial recovery when sulfamethiazine was administered, although his sleep disturbance has not resolved. He was able to return home after two weeks in hospital, but was lost for a follow-up.

Callicott et al. (1968) described a case of an eight-year-old boy in Virginia, USA, who presented with a mild abdominal pain and anorexia, followed by vomiting and fever, and a stiff neck. His physician administered one dose of penicillin G benzathine. The boy was admitted to the hospital. *Acanthamoeba* specimens were cultured from the cerebrospinal fluid and the boy was given ampicillin. He improved rapidly.

Most cases of primary amoebic meningoencephalitis were

reported in persons who had a history of swimming in lakes and swimming pools. However, a number of cases were also reported in persons with no history of swimming prior to the onset of the disease. Also, many strains of *Acanthamoeba*, including some with proven animal pathology, have been recovered from the noses and throats of human volunteers without clinical disease.

Wang and Fieldman (1967) isolated altogether 54 strains of free-living amoebas of the genus *Hartmanella* (= *Acanthamoeba*) in tissue cultures inoculated from pharyngeal swabs obtained for the study of viral respiratory diseases from 1958 to 1962 in families residing in two city-operated, low-cost apartment complexes. All cultures were examined microscopically for three to four weeks. Amoebas of the genus *Hartmanella* were demonstrated in 40 culture swabs from 38 subjects (two of them had positive cultures on two occasions). Most isolations (82%) were made from those four years of age and younger. Within this age range, children one and two years old contributed the majority of identified amoebas.

The relation between age and the occurrence of amoebas was analysed further by comparison of the ratios between actual and expected isolations within age groups. Again, children under five years of age were more apt to be affected, those under one year being most involved.

For some undetermined reason, isolation rates fell sharply during 1963-1966. However, some alterations in the swabbing and testing procedures and incubation temperature were made during this time because of changes in cell lines, contamination catastrophes and moving in a new building. Also, the study population during this period contained about eight per cent fewer youngsters in the group of one to four years old compared with the previous years.

**It is also quite well-established that amoebas are important contaminants of tissue cultures used in preparation of live biologicals, vaccines being the most important of them because they are widely injected into small babies and children.**

However, an alternative explanation is possible. In the above time interval, the US switched from the injectable Salk vaccine to the oral Sabin vaccine. It stands to reason that since amoebic forms are dissolved by bile, fewer specimens would pass into the body of the oral vaccine recipients compared with the recipients of the injectable polio vaccine.

The authors summarised that free-living amoebas are primarily found in soil and water, and it is reasonable to assume that they are acquired by man through oral contact with soil. This concept was supported by the large proportion of isolations from children in the crawling age group. But the explanation of two isolations from infants less than six months of age posed a problem. So, they concluded that they have not been successful in relating the presence of amoebas in the throat to the respiratory disease.

Again, at the time of this study, polio vaccination in the US was accepted by a great majority of parents and it is very likely that all babies in the age group below six months were vaccinated on time. This would plausibly as well as unequivocally explain the occurrence of amoebas in the throat swabs from the young babies.

About one third of the subjects had symptoms of respiratory disease at the time of positive cultures, but so did many others without amoebic isolations. Several attempts to measure antibody response to each of the species of amoeba ended in indecision.

The authors were unable to conclude that infection rather than infestation occurred or that immunity was acquired. The authors mentioned that amoebas were isolated originally from the monkey kidney tissue but did not consider a very likely administration of the polio vaccines as a possible source of amoebas in children below the age of four, one or even below the age of six months.

Apley et al. (1970) diagnosed meningoencephalitis due to an amoeba (*Naegleria*) in a little boy aged two years and nine months. He had one week's history of anorexia, mild irritability and slight sore throat. On the day of the admission, the baby complained of headache and more severe sore throat and was prescribed oral penicillin. He became progressively ill and vomited all food and liquids taken. Attacks of intermittent pallor and hot flushes were noticed by the parents. Lumbar puncture yielded a fluid, and amoebas were identified in it. The child progressively deteriorated and died on the sixteenth day of illness.

However, two other patients (Case 2 and Case 3) recovered. Case 2 was a six-year-old brother of Case 1. He suffered symptoms of meningoencephalitis and recovered 12 days after hospital admission. Amoebas were cultured from his CSF. Ten days after recovery, a further lumbar puncture yielded a normal CSF without any amoebas isolated.

Case 3 was a four years and five-months-old boy, a neighbour of cases 1 and 2. Two days before admission he had had a booster dose of DPT vaccines. Initially, amoebas were reported in his CSF; however, samples taken on the eighth, fourteenth and twenty-fourth days were all normal and no amoebas were isolated.

## OPTIC KERATOSIS CAUSED BY AMOEBAS

With the introduction of soft optic lenses, reports of eye keratosis started filling pages in medical journals. Amoebas were identified as the causative agent.

## SUMMARY

Amoebas are quite obviously widely spread protozoans and some of them have been established as causing serious disease in animals and humans. It is also quite well-established that amoebas are important contaminants of tissue cultures used in preparation of live biologicals, vaccines being the most important of them because they are widely injected into small babies and children.

Even the above short review of some of the existing literature indicates that a great number of cases of meningoencephalitis and serious respiratory infections in small babies and children can be linked to administration of vaccines which have a high potential to be contaminated by amoebas, either directly from the culture tissues or from culture tissues contaminated by amoebas from the air and surrounding laboratory surfaces.

It is also clear that although a number of victims recover, the majority succumb. Vaccine-producing drug companies should add warnings about this possibility on the product inserts of their vaccines and inform parents of the possibility of invasive amoebic meningoencephalitis.

This further highlights the need for prudence on the part of health authorities in Australia and elsewhere who, for yet another imperative reason, should drop their national immunisation programmes. ∞

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