

# Influenza Vaccination During Pregnancy: A Critical Assessment of the Recommendations of the Advisory Committee on Immunization Practices (ACIP)

---

David M. Ayoub, M.D.  
F. Edward Yazbak, M.D

---

## ABSTRACT

Influenza vaccination during all trimesters of pregnancy is now universally recommended in the United States. We critically reviewed the influenza vaccination policy of the CDC's Advisory Committee on Immunization Practice (ACIP) and the citations that were used to support their recommendations.

The ACIP's citations and the current literature indicate that influenza infection is rarely a threat to a normal pregnancy. There is no convincing evidence of the effectiveness of influenza vaccination during this critical period. No studies have adequately assessed the risk of influenza vaccination during pregnancy, and animal safety testing is lacking. Thimerosal, a mercury-based preservative present in most inactivated formulations of the vaccine, has been implicated in human neurodevelopment disorders, including autism, and a broad range of animal and experimental reproductive toxicities including teratogenicity, mutagenicity, and fetal death. Thimerosal is classified as a human teratogen.

The ACIP policy recommendation of routinely administering influenza vaccine during pregnancy is ill-advised and unsupported by current scientific literature, and it should be withdrawn. Use of thimerosal during pregnancy should be contraindicated.

On May 28, 2004, the Advisory Committee on Immunization Practice (ACIP) of the Centers for Disease Control and Prevention (CDC) published its annual report on its current policy for prevention of influenza.<sup>1</sup> The recommendation to vaccinate all pregnant women regardless of trimester was the most aggressive in a series of policy changes that began in 1995. Previously, influenza vaccine was advised only for women with preexisting medical conditions. The latest ACIP recommendation was promptly endorsed by the American College of Obstetricians and Gynecologists (ACOG) and the American Academy of Pediatrics (AAP).<sup>2,3</sup>

This investigation critically assesses the current ACIP recommendations, reviews the clinical research that supported them, and evaluates the risk-benefit analysis of administering inactivated influenza vaccine during pregnancy.

## Influenza Vaccine

Influenza vaccines are available in two forms: inactivated and live attenuated. Viruses for both vaccines are grown in embryonated hens' eggs, and therefore contain egg protein.

The attenuated live-virus vaccine is contraindicated during pregnancy. Clinicians should take care not to administer it inadvertently to a pregnant woman and also note that transmission of vaccine viruses to close contacts has occurred in clinical trials.<sup>4</sup>

The inactivated vaccine is available in two forms: the purified surface antigen preparation and a split-virus vaccine (Subviron, which is obtained by disrupting the virus using a non-ionic surfactant). Several methods are used to inactivate the viruses, and antibiotics are added to secure sterility. The split-virus vaccine is further purified chemically, and suspended in sodium phosphate-buffered isotonic sodium chloride solution.

Influenza vaccines available in the United States for the 2005-2006 influenza season are listed in Table 1. Most inactivated vaccines contain thimerosal, a mercury-containing compound (49.6% mercury by weight) that is rapidly metabolized to ethyl mercury. Influenza vaccines typically contain thimerosal at preservative levels of 0.01%, equivalent to 25 µg of mercury per 0.5 cc dose. Two forms of "preservative-free" vaccine packaged in single-dose presentations are available. One is manufactured without thimerosal (Fluzone, Sanofi-Aventis). In the other, thimerosal is removed at the end of the manufacturing process (Fluarix, GlaxoSmithKline). Almost all of the influenza vaccines administered to pregnant women in the 2005-2006 influenza season contained thimerosal at the preservative level.

## Influenza in Pregnancy

Influenza typically presents with constitutional (fever, myalgia, malaise, headache, fatigue) and upper respiratory (cough, sore throat, rhinitis) symptoms. Illnesses caused by the influenza virus are indistinguishable from those caused by a variety of other viral or nonviral pathogens. A definitive diagnosis requires laboratory testing (viral culture, rapid antigen testing, polymerase chain reaction, or immunofluorescence). An available rapid diagnostic test lacks the higher sensitivity and specificity of the other costlier and more time-consuming tests.

The virus is spread primarily through airborne transmission and direct contact with an infected individual. The incubation period is short (approximately two days), the onset of symptoms is abrupt, and the duration of the illness uncommonly exceeds one week. Complications including pneumonia, bronchitis, or sinusitis, or rarely encephalitis, transverse myelitis, Reye syndrome, myocarditis, or pericarditis, can occur at any age. More than 90% of influenza-related fatalities occur among the elderly.<sup>1</sup>

The ACIP's recent policy cites only two scientific papers to support its claim that influenza during pregnancy is more serious than at other times. A British study compared maternal and neonatal outcomes in women infected with the influenza virus during the second and third trimesters of pregnancy with those of pregnant, uninfected controls.<sup>5</sup> Only 11% of the 1,659 pregnant subjects had serological evidence of the illness; none had detectable influenza A-specific IgM. There was also no evidence for transplacental transmission of influenza virus, or autoantibody production in influenza-complicated pregnancies. Influenza infection had no significant impact on labor outcomes, health of the newborn, or maternal wellbeing.

**Table 1.** Influenza Vaccines Available to Pregnant Women in the United States for the 2005-2006 Influenza Season

| Name     | Type              | Manufacturer     | Available Doses | Thimerosal concentration | Mercury per dose | Indications |
|----------|-------------------|------------------|-----------------|--------------------------|------------------|-------------|
| Fluzone  | Inactivated Virus | Sanofi-Aventis   | 52-54 million   | 0.01%                    | 25µg/0.5ml       | ≥6 months   |
| Fluzone* | Inactivated Virus | Sanofi-Aventis   | 6-8 million     | 0                        | 0                | ≥6 months   |
| Fluvirin | Inactivated Virus | Chiron           | 18-26 million   | 0.01%                    | 25µg/0.5ml       | ≥4 years    |
| Fluarix  | Inactivated Virus | GlaxoSmith Kline | 7.5 million     | < 0.0005%                | <1.25µg/0.5ml    | ≥18 years   |

\*Thimerosal-free version, provided in two doses: 0.5cc (≥36 months age) and 0.25cc (6-35 months age)

The authors claimed that overall “complications” in pregnant women with influenza infection occurred more frequently than in controls (106/181 versus 73/180;  $P < .001$ ); however, no individual complication achieved statistical significance. Many of the listed complications appeared to be subjective complaints such as chest pain and “taking medication,” rather than specific diagnoses, and some could have been related to comorbid conditions that the authors failed to assess.

While there was only one recorded case of pneumonia during pregnancy, an uncommon but serious complication of influenza, all other “complications” lacked biological plausibility. When such nonspecific complications were excluded, there were no significant differences between the two groups. While the British research was cited in support of the ACIP’s aggressive influenza policy, it did not alter vaccination policy in the United Kingdom, where at present only pregnant women with serious preexisting medical conditions are vaccinated.<sup>6</sup>

In the second study cited in support of ACIP policy, Neuzil et al. reported that pregnant Medicaid-eligible women in a Tennessee registry had higher hospitalization rates during the influenza season than nonpregnant and postpartum women, particularly during the third trimester of pregnancy.<sup>7</sup>

Hospitalization was infrequent, ranging from 3.1 per 10,000 women-months in the first trimester to 10.5 per 10,000 in the third trimester. The study failed to discover a greater incidence of specific adverse events, including death, during pregnancies complicated by influenza. The following factors significantly limit the study’s impact:

- The Medicaid population is known to have more comorbidity and receive less adequate outpatient care. Patients are more likely to seek emergency room services at a more advanced stage of illness and thus to be admitted to the hospital more frequently.
- The hospitalization rate was overestimated by the inclusion of admissions for delivery.
- Influenza was not confirmed by laboratory testing and not differentiated from other infections.
- Reasons for admission were never quantified, and included a broad range of ICD-8 and ICD-9 codes.
- Aside from the event of a hospital admission, per se, no difference in morbidity or mortality was reported.

It is evident that neither study demonstrated that influenza infection during pregnancy was more serious than at other times.

A recent study that was not considered by the ACIP confirms the insignificance of influenza illness during pregnancy.<sup>8</sup> In that study of 49,585 pregnant women in a Kaiser Permanente HMO in Northern California, the influenza rate was 1.8 per 10,000. During five sequential influenza seasons there were only nine hospital admissions for pneumonia, fewer than two per season. All women

recovered. Predictably, the HMO hospitalization rate was much lower than that reported in the Medicaid population of Neuzil et al.<sup>7</sup> Viral identification was not performed; thus, it is likely that not all the women actually had influenza infections. Only 4.7% of the pregnant women had outpatient visits for influenza-like illness (ILI). This is lower than the 5 to 20% rate of influenza in the general population cited by the CDC.<sup>9</sup> Finally, the offspring’s hospital admission rate for pneumonia was only 0.35%.

The ACIP has stated there had been “excessive deaths” in pregnant women during prior influenza pandemics. The citations, however, contradict this conclusion. During the Asian flu pandemic of 1957-58 in a study from Johns Hopkins University, 55.6% (373/671) of an obstetrical population had “flu” symptoms during the outbreak, while 83.6% had measurable antibodies to influenza virus, suggesting that even in this indigent group of patients, a large number had a competent immune system and subclinical infections.<sup>10</sup> There was no statistically significant difference in fetal or maternal outcomes during the pandemic, and the authors concluded that outcomes did not differ from those in any of the prior four years.

A study reporting influenza in pregnant women during the 1918-1919 pandemic found that “no conclusions can be drawn, however, as to whether the incidence of influenza is greater among pregnant women than nonpregnant women ...”<sup>11</sup> Mortality was seen only in cases complicated by pneumonia, a condition easily treated today with antibiotics that were unavailable at that time. There were no deaths among the 672 cases of uncomplicated influenza. The 26% interruption of pregnancy rate with uncomplicated influenza was “not greatly in excess of the frequency one would expect under ordinary conditions” in 1918. Indeed, even in fatal cases, only 38% of the women experienced disruption of pregnancy, a testimony to the resilience of the developing fetus.

This is striking evidence that as far back as nearly a century, at a time when hygiene and medical care could not possibly be compared to the present, even the global pandemics of Spanish and Asian influenza did not threaten pregnancy outcomes. These studies also support the widely held belief that pregnancy is not a state of impaired immunity.

### Is Influenza Vaccination During Pregnancy Effective?

The ACIP reports a marginal impact of influenza vaccination during pregnancy.

“Researchers estimate that an average of one to two hospitalizations can be prevented for every 1,000 pregnant women vaccinated.”

The estimates cited by the ACIP are not supported in the current literature. A large study by Black and his associates at the Kaiser Permanente Northern California HMO, together with the Vaccine Safety Datalink Workgroup, a conglomeration of experts from the CDC and other institutions, was undertaken to assess the impact of influenza vaccination during pregnancy and the risk of ILI among mothers and their offspring.<sup>8</sup> Included in the review was information about 49,585 mothers and 48,639 live births for the November to February periods, from 1997 to 2002 inclusive.

There was no statistically significant difference in illness rates among the vaccinated and unvaccinated women (4.5/10,000 vs. 4.4/10,000) or their offspring. Vaccination also had no impact on illness rates among women with asthma (3.7/10,000 vs. 4.1/10,000), a subgroup the CDC has consistently claimed to be at high risk for influenza complications.

**Table 2.** CDC Data Regarding Efficacy of Influenza Vaccination

| Flu season | influenza virus culture-positive * | % antigenic match** | Maximum effectiveness*** |
|------------|------------------------------------|---------------------|--------------------------|
| 2004-2005  | 14.9 %                             | 40.1%               | 6.0%                     |
| 2003-2004  | 18.9%                              | 11.1%               | 2.1%                     |
| 2002-2003  | 11.6%                              | 98.7%               | 11.5%                    |
| 2001-2002  | 16.0%                              | 63.2%               | 10.1%                    |
| 2000-2001  | 11.0%                              | 57.7%               | 6.3%                     |
| mean       | <b>12.5%</b>                       | <b>54.2%</b>        | <b>7.2%</b>              |

\*specimens tested from World Health Organization (WHO) and National Respiratory and Enteric Surveillance System (NREVSS) collaborating laboratories

\*\* the antigenic match of lab specimen compared with vaccine antigens used that season

\*\*\*the likelihood that the causative agent of an influenza-like illness would have matched the vaccine antigen

Munoz et al. also failed to demonstrate effectiveness of influenza vaccination in pregnancy during five influenza seasons (1998-2003).<sup>12</sup> Rates of acute upper respiratory tract infection did not significantly differ between vaccinated (n = 225) and unvaccinated (n = 826) women (4.4% vs. 2.4%;  $P=2$ ). Paradoxically, the authors found four times as many ILI-related hospitalizations in vaccinated women (2.8% vs. 0.7%;  $P = 0.04$ ), an observation similar to that of Neuzil et al. (2.2% vs. 0.7%, OR 1.2).<sup>7</sup> These observations not only challenge vaccine effectiveness, but also raise concern that vaccination actually carries an added risk of ILI.

An analysis of these studies is a reminder that socioeconomic factors in influenza morbidity and mortality are frequently overlooked and may be of primary importance when considering a risk-benefit analysis for vaccination during pregnancy.

### The CDC's Influenza Statistics

In general, most symptoms of the “flu” are not caused by influenza virus but by a variety of noninfluenza viruses, bacteria, other infectious organisms, or even noninfectious conditions. According to the CDC, only about 20% of the cases of ILI are actually caused by the influenza virus.<sup>13</sup> If this is true, then theoretically only 20% of all cases of ILI are preventable by influenza vaccination, and only when there is a perfect antigenic match between the vaccine strain and the circulating virus. Furthermore, even a perfect antigenic match does not guarantee an adequate antibody titer, nor does measurable antibody assure protection.<sup>14</sup>

Table 2 summarizes viral surveillance data provided by the CDC from the last five influenza seasons.<sup>1-4,8</sup> On average, only 12.5% of samples submitted to collaborating laboratories in the United States identified influenza virus.

Because there are numerous strains of influenza virus and only three antigens can be chosen for the vaccine, a perfect match is unpredictable. According to the CDC, the average antigenic match from the previous five influenza seasons has been as low as 11.1%.<sup>1-3,9</sup> (Table 2). When we consider both variables of antigenic matching and the likelihood of laboratory confirmation of influenza virus, the average estimate of vaccine effectiveness is only a dismal 7.2%.

The CDC and news media frequently proclaim that there are about 36,000 influenza-associated deaths annually. Review of the mortality data from the CDC's National Vital Statistics System (NVSS) reveals these estimates are grossly exaggerated.<sup>2,6-9</sup> The NVSS reports preliminary mortality statistics and distinguishes

**Table 3.** Annual Influenza Death

| Year | Influenza and Pneumonia (all ages) | Influenza only (J10*&J11**) |                                 | Influenza virus identified (J10*) |                                 |
|------|------------------------------------|-----------------------------|---------------------------------|-----------------------------------|---------------------------------|
|      |                                    | All Ages                    | Reproductive Age Women (20-39y) | All Ages                          | Reproductive Age Women (20-39y) |
| 1997 | 86,449                             | 720                         | NA                              | NA                                | NA                              |
| 1998 | 91,871                             | 1,724                       | NA                              | NA                                | NA                              |
| 1999 | 63,730                             | 1,665                       | 10                              | 60                                | 0                               |
| 2000 | 65,313                             | 1,765                       | 13                              | 60                                | 1                               |
| 2001 | 62,034                             | 257                         | 5                               | 18                                | 1                               |
| 2002 | 65,681                             | 727                         | 3                               | 99                                | 1                               |

Source: National Vital Statistics System

\*J10=virus identified

\*\* J11=virus not identified

between influenza-related deaths and pneumonia-related mortality. When the final report is issued, influenza mortalities are combined with the far more frequent pneumonia deaths, yielding an exaggerated representation of “influenza” deaths. Pneumonia-related mortality due to immunosuppression, AIDS, malnutrition, and a variety of other predisposing medical conditions is therefore combined with seasonal influenza deaths. The actual influenza-related deaths for the years 1997 to 2002 ranged from 257 to 1,765 annually (Table 3). These values are further overestimated by combining deaths from laboratory-confirmed influenza infections with cases lacking laboratory confirmation. There were fewer than 100 annual cases of viral-confirmed deaths during this same period. Deaths occurring in women of reproductive ages were rare, approximately one per year.

### Is Influenza Vaccination Safe During Pregnancy?

Because the benefits of influenza vaccination during pregnancy appear lacking, a safety-benefit analysis should not tolerate any risk to vaccine recipients or their offspring, even at a theoretical level.

According to the ACIP, the safety of influenza vaccination during pregnancy is established in this way: “One study of influenza vaccination of >2,000 pregnant women demonstrated no adverse fetal effects associated with influenza vaccine.”<sup>1</sup>

This solitary safety study, by Heinonen et al., has in fact very little to do with the safety of influenza vaccination. The reported outcomes were strictly limited to malignancies, mostly after polio vaccination during pregnancy.<sup>3,1</sup>

The study's findings are important and alarming. Among 18,242 women who received the inactivated polio vaccine (IPV):

- The malignancy rate among 1-year-old children was nearly twice that of the unvaccinated control group (7.6 vs. 3.9 per 10,000;  $P < .05$ ).
- Neural tumor rate among the IPV recipients was 13 times greater than that of the unvaccinated (3.9 vs. 0.3 per 10,000;  $P < .01$ ).

The conclusions of the study were clear:

“The present data suggest that injection of killed polio vaccine in pregnant mothers were [*sic*] associated with malignancies, and tumors of neural origin in particular, in offspring born between 1959 and 1966.”

The publication contained only one sentence related to influenza vaccination outcomes:

“Among 2,291 mothers immunized with killed influenza vaccine during pregnancy, one child developed an astrocytoma of the spinal medulla.”

The narrow scope of the adverse events assessed, and the short period of pediatric follow-up (one year) severely limited the study's contribution to the very broad issue of influenza vaccination safety.

**Table 4.** Influenza Vaccine Mercury Dose Compared to EPA Allowable Daily Reference Dose

| Age            | Avg Weight | Max. Allowable Mercury* | Mercury From Flu Vaccine | Factor Over EPA Limit |
|----------------|------------|-------------------------|--------------------------|-----------------------|
| Pregnant adult | 70 kg      | 7.0 µg                  | 25 µg                    | 3.5 x                 |
| fetus          | 1 kg       | 0.01 µg                 | 25 µg                    | 200 x**               |
| 6 months       | 7.7 kg     | 0.77 µg                 | 12.5 µg                  | 16.2 x                |
| 12 months      | 10.5 kg    | 1.05 µg                 | 12.5 µg                  | 11.9 x                |
| 24months       | 12.3 kg    | 1.23 µg                 | 12.5 µg                  | 10.2 x                |
| 36 months      | 14.5 kg    | 1.45 µg                 | 25 µg                    | 17.2 x                |
| 4 years        | 16.3 kg    | 1.63 µg                 | 25 µg                    | 15.3 x                |
| 6 years        | 20.5 kg    | 2.05 µg                 | 25 µg                    | 12.2 x                |

\* based on the EPA daily reference dose of 0.1 micrograms/kg/day

\*\* based upon approximate maximum 4:1 preferential accumulation of mercury in cord blood

Nonmalignant adverse events, including harm to the fetus, were not recorded, and the lifetime risk of malignancy was not calculated.

Contrary to the ACIP's contention, this paper did not adequately assess the safety of influenza vaccination during pregnancy. It only raised serious concerns about polio vaccine safety, its link to cancers, and the bias of the ACIP. The study's findings should also reinforce the need for careful assessment of any medication given to pregnant women at a time of heightened fetal vulnerability to environmental exposures.

In addition, the Institute of Medicine Immunization Safety Review Committee recently rejected the validity of this paper after completing a review of the potential link between SV40 virus contamination of polio vaccines and cancer and concluded:

Because these epidemiologic studies are sufficiently flawed, the committee concluded in this report that the evidence was inadequate to conclude whether or not the contaminated polio vaccine caused cancer.<sup>3 2</sup>

The fact that the ACIP cited this study in support of the safety of influenza vaccination, while the Institute of Medicine rejected the research on the basis of flawed study design, is peculiar. There is a paucity of peer-reviewed reports on the safety of influenza vaccination in general, and more so on safety during pregnancy. Of the two pregnancy studies frequently cited, the first, by Sumaya and Gibbs, analyzed outcomes in only 56 women and did not assess infants beyond the immediate postnatal period.<sup>3 3</sup> The second study, by Deinard and Ogburn, included only 189 vaccinated pregnant women and followed the infants for only 6 to 8 weeks.<sup>3 4</sup> The fact that neither study revealed any adverse outcomes is not surprising, both being too small to detect infrequent birth defects or other adverse outcomes. Because of the very short follow-up, both studies were grossly inadequate in assessing complications including neurodevelopment disorders.

The more recent study by Munoz et al. exemplifies the all-too-frequent reality that many research studies are simply too poorly designed to support their conclusions.<sup>1 2</sup> That retrospective study examined data from five consecutive influenza seasons (1998-2003) in a large clinic in Houston, Texas. Only 252 pregnant women received the influenza vaccine. The authors reported: "...no serious adverse events occurred within 42 days of vaccination and there were no differences between groups [vaccinated versus unvaccinated] in the outcomes of pregnancy."

Because only infants who had at least one clinic visit were included, cases of fetal and neonatal demise would have been excluded. Vaccinated women demonstrated greater tendencies for abnormal glucose tolerance tests (8% vs. 4.5%;  $P=.05$ ), gestational diabetes (2.2% vs. 1.7%;  $P=.6$ ), and preeclampsia (4.8% vs. 3.9%;  $P=0.6$ ), as well as a significantly greater incidence of transient hypertension (6.7% vs. 2.9%;  $P<.01$ ). These unexpected findings

among vaccinated women may not be merely coincidental. Thimerosal-containing vaccinations have been linked to diabetes in other patient populations.<sup>3 5</sup> Mercury exposure has also been associated with hypertension.<sup>3 6</sup>

The authors concluded, "Influenza vaccine that was administered in the second or third trimester of gestation was safe in this study population." However, the authors themselves made it clear that the assessment of safety was limited and not targeted: The "evaluation of rare or long-term side effects of vaccination was not the goal of this study." This study did not adequately assess vaccine safety or justify the recommendation of influenza vaccination in the first trimester.

### Thimerosal, a Preservative with Risks

Most influenza vaccines given in the United States during the 2005-2006 influenza season contained thimerosal. In the late 1980s, the number of routine childhood immunizations, most containing thimerosal, began to increase.

As a result of the concerns that rising mercury exposures could exceed regulatory guidelines and pose health risks, a joint statement was issued by the American Academy of Pediatrics and the Public Health Service on July 7, 1999, and endorsed by the American Academy of Family Physicians. The policy statement established the goal of removing thimerosal from vaccines routinely recommended for infants "as soon as possible."<sup>7</sup> The ACIP influenza report reassuringly stated that there "was no scientific evidence that thimerosal in vaccines leads to serious adverse events." In failing to insist on the use of thimerosal-free influenza vaccinations during pregnancy, as well as in early childhood, the ACIP policy changes will again result in increasing mercury exposures to the most susceptible individuals.

Because a risk-benefit analysis of influenza vaccination during pregnancy cannot ignore the potential implications of mercury exposure during the vulnerable prenatal period, a brief review of thimerosal is prudent.

Thimerosal is an ethyl mercury-thiosalicylate compound that rapidly disassociates into ethyl mercury, a short-chain alkyl mercurial. It has also been shown that ethyl mercury is further metabolized to inorganic mercury.<sup>3 8</sup> Therefore, toxicity data from studies of thimerosal, ethyl mercury, and inorganic mercury are all relevant in assessing influenza vaccine safety.

There are currently no federal regulatory guidelines for ethyl mercury exposure by injection; however, the Environmental Protection Agency (EPA) has established guidelines for ingested methylmercury, which has been far more extensively studied. The EPA's current daily allowable methylmercury exposure limit is 0.1 µg/kg/day. Both forms of mercury are structurally similar short-chain alkyl (organic) mercurials that readily enter cell membrane lipid bilayers, including the placenta and brain.<sup>3 9 4 0</sup>

Most adult influenza vaccines contain an equivalent of 25 µg of mercury per dose (Table 1). An average-sized pregnant woman receiving an influenza vaccine will be exposed to organic mercury that exceeds the EPA limit by a factor of 3.5 (Table 4). The fetus could potentially receive a dose of mercury that exceeds EPA limits by a much larger factor. Furthermore, fetal blood mercury concentrations have been shown to be as much as 4.3 times the maternal level.<sup>4 1</sup> A larger proportion of ethyl mercury accumulates in fetal tissues relative to maternal tissues, especially in the central nervous system.<sup>4 2</sup> The observation of a 7.8-15.7% prevalence of elevated umbilical cord mercury in the United States, at levels associated with loss of IQ, adds to the significance of additional mercury exposure from prenatal vaccination.<sup>4 3</sup>

**Table 5.** Fetal Death Rates (Abortion, Resorption, and Stillbirth) from Prenatal Exposure to Thimerosal in Several Species and by Various Administration Routes.

| Author                            | Prenatal Exposure | Route                | Species      | Offspring (n) | Fetal Death Rate | Relative Risk |
|-----------------------------------|-------------------|----------------------|--------------|---------------|------------------|---------------|
| Gasset et al., 1975 <sup>61</sup> | 2% thimerosal     | intraperitoneal      | rat          | 109           | 35.8%            | 32.5 x        |
|                                   | 0.2% thimerosal   | intraperitoneal      | rat          | 125           | 13.6%            | 12.4 x        |
|                                   | saline            | intraperitoneal      | rat          | 95            | 1.1%             |               |
|                                   | 2% thimerosal     | conjunctival         | rabbit       | 56            | 39.3%            | 2.6 x         |
|                                   | saline control    | conjunctival         | rabbit       | 39            | 15.4%            |               |
| Itoi et al., 1972 <sup>62</sup>   | 0.2% thimerosal   | conjunctival         | rabbit       | 22            | 18.2%            | 5.2 x         |
|                                   | 0.02% thimerosal  | conjunctival         | rabbit       | 68            | 16.2%            | 4.6 x         |
|                                   | saline control    | conjunctival         | rabbit       | 86            | 3.5%             |               |
| Digar et al., 1987 <sup>63</sup>  | 0.1 mg thimerosal | injected in yolk sac | chick embryo | 254           | 46.5%            | 5.1 x         |
|                                   | unexposed control | none                 | chick embryo | unreported    | 9.1%             |               |

## Neurotoxicity of Mercury

In spite of the ACIP claims, concerns about potential neurotoxicity of thimerosal are widely reported by various health authorities. The Eli Lilly Manufacturer's Safety Data Sheet (MSDS) states: "exposure in-utero can cause mild to severe mental retardation and motor coordination impairment." The National Toxicology Program (NTP) states that thimerosal is a "poison by ingestion, subcutaneous, intravenous and possibly other routes," classifies it as an experimental carcinogen and teratogen, and concludes that childhood exposures result in "mental retardation in children, loss of coordination in speech, writing, gait, stupor, and irritability and bad temper progressing to mania."<sup>44</sup>

In recent years there has been a growing body of science that has linked thimerosal exposure to neurodevelopment disorders (NDs), including autism and attention deficit/hyperactivity disorder. These studies have brought forth toxicologic, biochemical, experimental, neuroimmunologic, and epidemiologic evidence.<sup>3, 8, 5, 8</sup> A comprehensive discussion of this science is beyond the scope of this paper and has been reviewed elsewhere.<sup>5, 9</sup>

Because the ACIP influenza policy endorses exposure of the fetus to ethyl mercury, any studies demonstrating harm from prenatal ethyl mercury exposure must be carefully reviewed. Unfortunately, there is only one known published study that attempted to correlate prenatal thimerosal exposure to NDs. Holmes et al. determined that mothers of autistic children received nearly six times more thimerosal-preserved Rho D immuno-globulin than mothers of neurotypical children (0.53 vs. 0.09 mean shots;  $P < 0.0000004$ ), strongly implying a role of prenatal mercury exposure in adverse developmental outcomes.<sup>4, 5</sup>

## Fetal and Reproductive Toxicity of Mercury

The 2005-2006 Fluzone, Fluvirin, and Fluarix package inserts clearly state that animal reproductive safety studies have not been conducted during pregnancy and that risks to the human fetus were never investigated, including mutagenicity, carcinogenicity, and effects on future fertility. The Fluzone manufacturer states that the vaccine should be given to pregnant women only if clearly needed. The Fluvirin insert adds that the clinical judgment of the attending physician should prevail. The Fluarix insert only affirms the ACIP recommendation.

The manufacturer of the live vaccine FluMist issues a similar warning: "Animal reproduction studies have not been conducted with FluMist. It is also not known whether FluMist can cause fetal harm when administered to a pregnant woman or affect reproduction capacity." The manufacturer is careful to add: "Therefore, FluMist should not be administered to pregnant women."

The Eli Lilly MSDS further states that thimerosal "is known to cause birth defects and other reproductive harm." The NTP broadly classifies thimerosal as a teratogen capable of other adverse reproductive effects.<sup>44</sup> The California EPA has proclaimed that thimerosal is a human reproductive toxin. When denying a request from Bayer, Inc., to reclassify thimerosal as harmless, its report concluded:

The scientific evidence that... thimerosal causes reproductive toxicity is clear and voluminous. Thimerosal dissociates in the body to ethyl mercury. The evidence for its reproductive toxicity includes severe mental retardation or malformations in human offspring who were poisoned when their

mothers were exposed to ethyl mercury or thimerosal while pregnant, studies in animals demonstrating developmental toxicity after exposure to either ethyl mercury or thimerosal, and data showing interconversion to other forms of mercury that also clearly cause reproductive toxicity.<sup>6, 0</sup>

The peer-reviewed literature summarized in Table 5 raises additional concerns about prenatal thimerosal exposure. Gasset et al. observed significantly more fetal deaths after maternal thimerosal exposure compared with control animals, indicating that, even topically, thimerosal had abortifacient qualities.<sup>61</sup> These findings were replicated by Itoi et al., who demonstrated a nearly five-fold greater fetal death rate when topical thimerosal solution was applied to the conjunctiva of pregnant female rabbits.<sup>62</sup> Fetal malformations occurred more frequently in thimerosal-exposed vs. saline controls (9.1% vs. 0.0%). Digar et al. discovered a four-fold increased mortality rate when the yolk sacs of chicks were injected with 0.1 mg of thimerosal.<sup>63</sup> Gross malformations occurring in 36% of exposed embryos but in none of the controls included syndactyly, visceroptosis, thinning of the abdominal wall, and limb and wing growth abnormalities.

Thimerosal also has the potential to impair fertility. Batts et al. demonstrated that thimerosal was toxic to cilia function when applied topically to the trachea of sheep, indicating a potential biologic mechanism for damaging reproductive capacity in women (fallopian tube) and men (sperm motility).<sup>64</sup> This could explain the observation that adult survivors of childhood mercury poisoning (acrodynia from infantile teething powder) have a greater incidence of infertility.<sup>65</sup> Goncharuk discovered a significant dose-dependent rate of fetal death in rats and mice exposed to ethyl mercury compounds by inhalation. When ethyl mercury was given orally to albino rats prior to mating, diminished fertility was observed, not only in adult recipients but also in first and second-generation progeny.<sup>66</sup>

A comprehensive discussion of the reproductive toxicity of inorganic mercury, another thimerosal metabolite, is beyond the scope of this review. Inorganic mercury has been shown to be a genotoxin and a reproductive toxin in various animal and in-vitro systems.<sup>6, 7</sup> Khan et al., for example,

demonstrated fertility and survival reduction in mice exposed to mercuric chloride.<sup>6</sup> \*These effects, including ovarian atrophy, were seen in the absence of overt mercury toxicity, underscoring the need for carefully designed clinical studies assessing the risk of prenatal thimerosal exposure.

Human studies designed to assess the potential reproductive toxicity of thimerosal are sparse. Heinonen, the lead author of one of the previously cited ACIP influenza vaccine "safety studies," confirmed human reproductive toxicity of thimerosal in a different publication.<sup>6</sup> \*Using data from the Collaborative Perinatal Project that was sponsored by the FDA, U.S. Public Health Service, and the National Institutes of Health, the researchers showed that topical thimerosal exposure during pregnancy significantly increased risks for human birth defects.

The human reproductive and fetal toxicity of methylmercury has been widely studied and accepted. Many agencies, including the CDC and FDA, proclaim that methylmercury is more toxic than ethyl mercury, but this is not supported in the scientific literature. For example, in an experimental study of swine, researchers found ethyl mercury to be significantly more toxic than methylmercury.<sup>7</sup> \*0 Jacquet and Laureys reported that ethyl mercury crossed the placenta more readily than methylmercury and was capable of mutagenicity in the form of induction of C-mitosis in eukaryotes and HeLa cells, resulting in aneuploidy or polyploidy.<sup>7</sup> \*1Sex-linked recessive lethals were reported in *Drosophila melanogaster*.

Coupling the incontrovertible evidence of the experimental reproductive toxicity of thimerosal and its metabolites to the limited scope of available human safety studies, it is astonishing that the ACIP's recommendation to administer the influenza vaccine during pregnancy has not been previously challenged. The omission of these known risks of a major influenza vaccine component from the package inserts would imply that the drug is clearly mislabeled.

## Conclusions

The ACIP's recommendation of influenza vaccination during pregnancy is not supported by citations in its own policy paper or in current medical literature. Considering the potential risks of maternal and fetal mercury exposure, the administration of thimerosal during pregnancy is both unjustified and unwise. Pregnancy should continue to be a time when doctors are highly protective of their patients with regard to any fetal exposure. Without adequate safety testing, a risk-benefit analysis of influenza vaccination during pregnancy is not possible, and therefore the ACIP's present recommendation should be withdrawn.

**David M. Ayoub, M.D.**, is Medical Director of Prairie Collaborative, Ltd. Contact: Memorial Medical Center, 710 North First St., Springfield, IL 62781, raypoke@mac.com. Tel: (217) 788-7021, Fax: (217) 788-5588. **F. Edward Yazbak, M.D., FAAP**, is the founder of TL Autism Research, Falmouth, MA.

## REFERENCES

- 1 Prevention and Control of Influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 2004;53(May 28, RR6):1-44.
- 2 American College of Obstetrics and Gynecology. ACOG Committee Opinion No. 305, November 2004. Influenza vaccination and treatment during pregnancy. *Obstet Gynecol* 2004;104:1125-1126.
- 3 American Academy of Pediatrics and American College of Obstetricians and Surgeons. *Guidelines for Perinatal Care*, 5th ed; 2002;92.
- 4 CDC. Questions and answers: the nasal-spray flu vaccine. Updated Sept 13, 2005. Available at: [www.cdc.gov/flu/about/qa/nasalspray.htm](http://www.cdc.gov/flu/about/qa/nasalspray.htm). Accessed Mar 6, 2006.

- 5 Irving WL, James DK, Stephenson T, et al. Influenza virus infection in the second and third trimesters of pregnancy: a clinical and seroepidemiological study. *Br J Obstet Gynaecol* 2000;107:1282-1289.
- 6 Department of Health, Scottish Executive Health Department, Welsh Assembly Government. Chapter 20, Influenza. Immunisation against infectious disease. In: *The "Green Book."* United Kingdom: Department of Health; 2005:1-16.
- 7 Neuzil KM, Reed GW, Mitchel EF, Simonsen L, Griffin MR. Impact of influenza on acute cardiopulmonary hospitalizations in pregnant women. *Am J Epidemiol* 1998;148:1094-1102.
- 8 Black SB, Shinefield HR, France EK, et al. Effectiveness of the influenza vaccine during pregnancy in preventing hospitalizations and outpatient visits for respiratory illness in pregnant women and their infants. *Am J Perinatol* 2004;21:333-339.
- 9 CDC and Prevention. Key facts about influenza and the influenza vaccine. Updated Sept 28, 2005. Available at: [www.cdc.gov/flu/keyfacts.htm](http://www.cdc.gov/flu/keyfacts.htm). Accessed April 22, 2006.
- 10 Hardy JMB, Azarowicz EN, Mannini A, Medearis DN, Cooke RE. The effect of Asian flu on the outcome of pregnancy, 1957-1958. *Am J Pub Health* 196;51:1182-1188.
- 11 Harris JW. Influenza occurring in pregnant women. A statistical study of thirteen hundred and fifty cases. *JAMA* 1919;72:978-980.
- 12 Munoz FM, Greisinger AJ, Wehmanen OA, et al. Safety of influenza vaccination during pregnancy. *Am J Obstet Gynecol* 2005;192:1098-1106.
- 13 U.S. Food and Drug Administration. Transcript of the Vaccines and Related Biological Advisory Committee, Feb 20, 2003. Available at: [www.fda.gov/ohrms/dockets/ac/03/transcripts/3923t1.htm](http://www.fda.gov/ohrms/dockets/ac/03/transcripts/3923t1.htm). Accessed Apr 22, 2006.
- 14 Del Giudice G, Podda A, Rappuoli R. What are the limits of adjuvanticity? *Vaccine* 2001;15(20 Suppl 1):S38-S41.
- 15 CDC. Update: Influenza activity—United States and worldwide, 2000-01 season, and composition of the 2001-02 influenza vaccine. *MMWR* 2001;50:466-470.
- 16 CDC. Update: Influenza activity—United States and worldwide, 2001-02 season, and composition of the 2002-03 influenza vaccine. *MMWR* 2002;51:503-506.
- 17 CDC. Update: Influenza activity—United States and worldwide, 2002-03 season, and composition of the 2003-04 influenza vaccine. *MMWR* 2003;52:516-521.
- 18 CDC. Update: Influenza activity—United States and worldwide, 2003-04 season, and composition of the 2004-05 influenza vaccine. *MMWR* 2004;53:547-552.
- 19 CDC. Update: Influenza activity—United States and worldwide, 2004-05 season, and composition of the 2005-06 influenza vaccine. *MMWR* 2005;54:631-634.
- 20 Kochanek KD, Smith BL. Deaths: Preliminary Data for 2002. *National Vital Statistics Reports* 2004;51(13):1-48.
- 21 Hoyert DL, Kochanek KD, Murphy SL. Deaths: final data for 1997. *National Vital Statistics Reports* 1999;47(19):1-105.
- 22 Murphy SL. Deaths: final data for 1998. *National Vital Statistics Reports* 2000;48(11):1-106.
- 23 Hoyert DL, Arias E, Smith BL, Murphy SL, Kochanek KD. Deaths: final data for 1999. *National Vital Statistics Reports* 2001;49(8):1-114.
- 24 Miniño AM, Arias E, Kochanek KD, Murphy SL, Smith BL. Deaths: final data for 2000. *National Vital Statistics Reports* 2002;50(15):1-120.
- 25 Arias E, Anderson RN, Hsiang-Ching K, Murphy SL, Kochanek KD. Deaths: final data for 2001. *National Vital Statistics Reports* 2003;52(3):1-116.
- 26 Kochanek KD, Murphy SL, Anderson RN, Scott C. Deaths: final data for 2002. *National Vital Statistics Reports* 2004;53(5):1-105.
- 27 CDC/NCHS. National Vital Statistics System. Mortality. GMWK 1 Worktable 1. Deaths from each cause by 5-year age groups, race and sex. United States 1999. Available at: <http://198.246.96.2/nchs/datawh/statab/unpubd/mortabs/gmwiki10.htm>. Accessed Dec 31, 2005.

- <sup>28</sup> CDC/NCHS. National Vital Statistics System. Mortality. GMWK I Worktable 1. Deaths from each cause by 5-year age groups, race and sex. United States 2000. Available at: <http://198.246.96.2/nchs/datawh/statab/unpubd/mortabs/gmwki10.htm>. Accessed Dec 31, 2005.
- <sup>29</sup> CDC/NCHS. National Vital Statistics System. Mortality. GMWK I Worktable 1. Deaths from each cause by 5-year age groups, race and sex. United States 2001. Available at: <http://198.246.96.2/nchs/datawh/statab/unpubd/mortabs/gmwki10.htm>. Accessed Dec 31, 2005.
- <sup>30</sup> CDC/NCHS. National Vital Statistics System. Mortality. GMWK I Worktable 1. Deaths from each cause by 5-year age groups, race and sex. United States 2002. Available at: <http://198.246.96.2/nchs/datawh/statab/unpubd/mortabs/gmwki10.htm>. Accessed Dec 31, 2005.
- <sup>31</sup> Heinonen OP, Shapiro S, Monson RR, et al. Immunization during pregnancy against poliomyelitis and influenza in relation to childhood malignancy. *Int J Epidemiol* 1973;2:229-235.
- <sup>32</sup> Stratton K, Almario DA, and McCormick MC, eds. *Immunization Safety Review: SV40 Contamination of Polio Vaccine and Cancer*. Washington, D.C.: National Academies Press; 2002. Available at: [www.iom.edu/CMS/3793/4705/4317.aspx](http://www.iom.edu/CMS/3793/4705/4317.aspx). Accessed Mar 1, 2006.
- <sup>33</sup> Sumaya CV, Gibbs RS. Immunization of pregnant women with influenza A/New jersey/76 virus vaccine: reactogenicity and immunogenicity in mother and infant. *J Infect Dis* 1979;140:141-146.
- <sup>34</sup> Deinard AS, Ogburn P. A/NJ/8/76 influenza vaccination program: effects on maternal health and pregnancy outcome. *Am J Obstet Gynecol* 1981;140:240-245.
- <sup>35</sup> Wahlberg JW, Fredrikson J, Virrala O, Ludvigsson J: Abis Study Group. Vaccinations may induce diabetes in one-year-old children. *Ann NY Acad Sci* 2003;1005:404-408.
- <sup>36</sup> Pedersen EB, Jorgensen ME, Pedersen MB, et al. Relationship between mercury in blood and 24-h ambulatory blood pressure in Greenlanders and Danes. *Am J Hypertens* 2005;18:612-618.
- <sup>37</sup> Joint Statement of the American Academy of Pediatrics (AAP) and the United States Public Health Service (USPHS). *Pediatrics* 1999;104:568-569.
- <sup>38</sup> Burbacher TM, Shen DD, Liberato N, et al. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal. *Environ Health Perspect* 2005;113:1015-1021.
- <sup>39</sup> Goldman LR, Shannon MW. Committee on Environmental Health. Technical report: Mercury in the environment and implications for pediatricians. *Pediatrics* 2001;108:197-205.
- <sup>40</sup> Slikker W Jr. Developmental neurotoxicity of therapeutics: survey of novel recent findings. *Neurotoxicology* 2000;21:50.
- <sup>41</sup> U.S. Environmental Protection Agency Integrated Risk Information System. Reference dose for chronic oral exposure (RfD). In: *Methylmercury (MeHg) (CASRN 22967-92-6)* Available at: [www.epa.gov/iris/subst/0073.htm](http://www.epa.gov/iris/subst/0073.htm). Accessed Apr 22, 2006.
- <sup>42</sup> Ukita, T, Takeda Y, Sato Y, Takahashi T. Distribution of 203Hg labeled mercury compounds in adult and pregnant mice determined by whole-body autoradiography. *Radioisotopes* 1967;16:439-448.
- <sup>43</sup> Trasande L, Landrigan PJ, Schechter C. Public health and economic consequences of methylmercury toxicity to the developing brain. *Environ Health Perspect* 2005;113:590-596.
- <sup>44</sup> National Institute of Environmental Health Science. *Thimerosal [54-6-8] Nomination to the National Toxicology Program. Review of the Literature*. Available at: [http://ntp.niehs.nih.gov/ntp/htdocs/Chem\\_Background/ExSumPDF/Thimerosal.pdf](http://ntp.niehs.nih.gov/ntp/htdocs/Chem_Background/ExSumPDF/Thimerosal.pdf). Accessed Mar 1, 2006.
- <sup>45</sup> Holmes AS, Blaxill MF, Haley BE. Reduced levels of mercury in first baby haircuts of autistic children. *Int J Toxicol* 2003;22:277-285.
- <sup>46</sup> Bradstreet J, Geier DA, Kartzinell JJ, Adams JB, Geier MR. A case-control study of mercury burden in children with autistic spectrum disorders. *J Am Phys Surg* 2003;8:76-79.
- <sup>47</sup> James SJ, Slikker W 3rd, Melnyk S, et al. Thimerosal neurotoxicity is associated with glutathione depletion: protection with glutathione precursors. *Neurotoxicology* 2005;26:1-8.
- <sup>48</sup> James SJ, Cutler P, Melnyk S, et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *Am J Clin Nutr* 2004;80:1611-1617.
- <sup>49</sup> Waly M, Olteanu H, Banerjee R, et al. Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal. *Mol Psychiatry* 2004;9:358-370.
- <sup>50</sup> Hornig M, Chian D, Lipkin WI. Neurotoxic effects of thimerosal are mouse strain dependent. *Mol Psychiatry* 2004;9:833-845.
- <sup>51</sup> Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol* 2005;57:67-81.
- <sup>52</sup> Havarinasab S, Haggqvist B, Bjorn E, Pollard KM, Hultman P. Immunosuppressive and autoimmune effects of thimerosal in mice. *Toxicol Appl Pharmacol* 2005;204:109-121.
- <sup>53</sup> Geier MR, Geier DA. Thimerosal in childhood vaccines, neurodevelopment disorders, and heart disease in the United States. *J Am Phys Surg* 2003;8:6-11.
- <sup>54</sup> Geier MR, Geier DA. Neurodevelopmental disorders after thimerosal-containing vaccines: a brief communication. *Exp Biol Med* 2003;228:660-664.
- <sup>55</sup> Geier DA, Geier MR. Neurodevelopmental disorders following thimerosal-containing vaccines: a follow-up analysis. *Int J Toxicol* 2004;23:369-76.
- <sup>56</sup> Geier DA, Geier MR. A comparative evaluation of the effects of MMR immunization and mercury doses from thimerosal containing childhood vaccines on the population prevalence of autism. *Med Sci Monit* 2004;10:133-139.
- <sup>57</sup> Geier DA, Geier MR. A two-phased population epidemiological study of the safety of thimerosal-containing vaccines: a follow-up analysis. *Med Sci Monit* 2005;11:160-170.
- <sup>58</sup> Geier DA, Geier MR. Early downward trends in neurodevelopmental disorders following removal of thimerosal-containing vaccines. *J Am Phys Surg* 2006;11:8-13.
- <sup>59</sup> Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? *Neuroendocrinol Lett* 2005;26:439-446.
- <sup>60</sup> Office of Environmental Health Hazard Assessment. Response to the Petition of Bayer Corporation for Clarification of the Proposition 65 Listing of "Mercury and Mercury Compounds" as Chemicals Known to Cause Reproductive Toxicity; February 2004. Available at: [www.oehha.ca.gov/prop65/cnrn\\_notices/hgbayer.html](http://www.oehha.ca.gov/prop65/cnrn_notices/hgbayer.html). Accessed Mar 1, 2006.
- <sup>61</sup> Gassett AR, Itoi M, Ishii Y, Ramer RM. Teratogenicities of ophthalmic drugs. II. Teratogenicities and tissue accumulation of thimerosal. *Arch Ophthalmol* 1975;93:52-55.
- <sup>62</sup> Itoi M, Ishii Y, Kaneko N. Teratogenicities of antiviral ophthalmics on experimental animals. *Jpn J Clin Ophthal* 1972;26:631-640.
- <sup>63</sup> Digar A, Senshama GC, Samal SN. Lethality and teratogenicity of organic mercury (thimerosal) on the chick embryo. *J Anat Soc India* 1987;36:153-159.
- <sup>64</sup> Batts AH, Marriott C, Martin GP, Wood CF, Bond SW. The effect of some preservatives used in nasal preparations on mucus and ciliary components of mucociliary clearance. *J Pharm Pharmacol* 1990;42:145-151.
- <sup>65</sup> Dally A. The rise and fall of pink disease. *Soc Hist Med* 1997;10:291-304.
- <sup>66</sup> Goncharuk GA. Experimental investigation of the effect of organomercury pesticides on generative function and on progeny. *Hyg Sanit* 1971;36:40-44.
- <sup>67</sup> Clement Associates. *Toxicological Profile for Mercury*. Atlanta, Ga.: Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, U.S. Public Health Services; 1989.
- <sup>68</sup> Kahn AT, Atkinson A, Graham TC, et al. Effects of inorganic mercury on reproductive performance of mice. *Food Chem Toxicol* 2004;42:571-577.
- <sup>69</sup> Heinonen OP, Slone D, Shapiro S. *Birth Defects and Drugs in Pregnancy*. Littleton, Mass.: Publishing Sciences Group; 1976.
- <sup>70</sup> Tyrphonas L, Nielsen NO. Pathology of chronic alkylmercurial poisoning in swine. *Am J Vet Research* 1973;34:379-392.
- <sup>71</sup> Leonard A, Jacquet P, Lauwerys RR. Mutagenicity and teratogenicity of mercury compounds. *Mutat Res* 1983;114:1-18.