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Control of Avian Encephalomyelitis: A Historical Account

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SUMMARY. Avian encephalomyelitis control methods were not developed until the 1950s although the disease had been discovered and described over 20 yr earlier. Inability to transmit the infection by other than intracerebral inoculation, lack of suitable immunologic methods, the unknowing use of immune chickens or embryos for experimental studies, and reliance on a highly adapted strain of virus rather than fresh field isolates were the main reasons for a general lack of progress. In the absence of supportive experimental data, at least two commercial breeding organizations turned to the use of a crude chicken brain–propagated virus for vaccination of breeder replacement flocks in the 1950s. This control procedure turned out to be practical and efficacious. Development of suitable embryo infection methods and immunologic tests and the chance finding that antibody-free flocks were essential for experimental studies led to the development of embryo-susceptibility tests to identify immune breeder flocks and formed the basis for another commercially applied control program, the testing and selection of only immune flocks for hatching purposes. The application of the new testing methods coupled with a switch from the adapted Van Roekel strain of virus to fresh field isolates for experimentation resulted in a rapid unraveling of the epizootiology and pathogenesis of the disease and also to the development of a safe and effective vaccine that was licensed for administration to breeder replacements in 1962.

Key words: encephalomyelitis, history, chickens, vaccine, picornavirus

Abbreviations: AE = avian encephalomyelitis; CNS = central nervous system; EID_{50} = embryo infective dose–50%; H&N = Heisdorf and Nelson; IM = intramuscular; SPF = specific-pathogen-free; USDA = United States Department of Agriculture; WW = wing web

If avian encephalomyelitis (AE) were to have arisen as a problem in the present decade, control probably would have been effected very rapidly. Infection of chickens and certain other avian species by the causative picornavirus is uncomplicated and is easily detected by a variety of serologic means; furthermore, it is self-limiting, and a simple humoral immune response provides lifelong protection (14). However, many of the requisite research tools were not available at the time AE was discovered in the early 1930s, and certain epizootiologic features of the disease were perplexing. Because the disease was a burden primarily on the poultry breeding companies, there was not a universal clamor for a crash program to address the issue, and research was carried forward by a relatively small number of investigators. Consequently, it took over 20 yr for effective control methods to begin to emerge, and another decade passed before an effective licensed vaccine was available. This review focuses on the early history of the disease and the work that led to its control.

THE DISEASE: ITS IDENTIFICATION AND EARLY DESCRIPTION

AE, a viral disease of chickens, turkeys, pheasants, and quail, was first described by Dr. E. Elizabeth Jones, who was associated with the Harvard Medical School and School of Public Health in Boston. She published two papers on the disease in 1932 and 1934. The first was a preliminary report (28) in which she described "an encephalomyelitis in the chicken" characterized by rapid tremor of the head and neck, sometimes associated with ataxia. The initial case consisted of nine affected chicks submitted for diagnosis from a flock in Massachusetts in May 1930, and an additional three affected

flocks were seen by May 1932, all from Massachusetts. The report described microscopic lesions of the central nervous system (CNS) and also the successful transmission of the disease to normal chickens by intracranial inoculation of brain or spinal cord suspension collected from affected chickens. Dr. Jones's second paper (29) detailed field and laboratory studies of the disease, which had become increasingly more prevalent since the initial report. This report included a more detailed account of the histopathologic lesions and their distribution (with credit to Dr. M. M. Canavan of the Department of Pathology at Harvard for the original description of the CNS lesions), along with observations on the epidemiology, etiology, and transmission of the disease. Many important features of the condition were reported, e.g., its appearance in some chicks within 2–3 days after hatching but in most birds after about 3 wk of age, its occurrence primarily in the first 1 to 3 hatches of the season, and its absence in adult chickens. Also, the etiology was determined to be a relatively stable filterable agent (virus) that could be serially passaged by intracerebral inoculation of brain or spinal cord. Egg transmission of the virus was suspected by Jones (28) but not proven.

The name "epidemic tremor" was applied to reflect the widespread occurrence of the disease within a flock and the fact that affected chicks often first displayed a trembling of the head and neck. However, Henry Van Roekel and colleagues at the University of Massachusetts (51) later noted that tremor was not always seen, and when it was observed, it followed the appearance of ataxia. Because of the infectious nature of the disease and the fact that it mainly affected the CNS, they proposed the term "infectious avian encephalomyelitis," a name later shortened to avian encephalomyelitis and adopted by a committee of the American Veterinary Medical Association in 1939 (5).

EARLY EPIZOOTIOLOGIC STUDIES

Early studies and reports of the disease came exclusively from New England. In addition to the reports from Jones, C. A. ("Cab") Bottorff (6,7) recorded an outbreak on the University of New Hampshire Poultry Farm in 1932. By 1934, he had found eight outbreaks in New Hampshire, three in Maine, and one in Vermont. Jones (28) also mentioned these outbreaks plus one in Connecticut. Van Roekel, along with his colleagues Kenneth Bullis and Miriam Clarke, noted a rising prevalence of the disease in Massachusetts in the mid 1930s (5) as did Erwin Jungherr and Edwin Minard in Connecticut (30). The latter authors also cited numerous reports or personal communications showing that by 1942 the disease had been observed not only throughout the New England states but also in other northeastern, southern and western states, and even as far away as Australia. The infection is now known to be worldwide and to affect pheasants, quail and turkeys as well as chickens (14,45,50).

Whether the disease in chickens occurred *de novo* in Massachusetts and then spread to other areas or was simply recognized elsewhere once it had been described in the literature has never been established, although it is clear that spread was a major factor in its increasing prevalence over the 20-yr period between 1930 and 1950. An example is an outbreak of AE at Kimber Farms, Inc. in California that could be attributed to the importation of hatching eggs from the New England area in 1941 (41). Of course it is possible that the disease was present in some areas long before it was first reported but was either overlooked or confused with other neurologic conditions. It is also likely that, if present, the infection often failed to provoke clinical signs because of the lack of the specific conditions required for its appearance in chicks. For instance, even if virus was present in an area, it probably would not be evident unless the breeding stock was sufficiently isolated. In well isolated flocks, first exposure often was delayed until after egg production commenced when infection could result in egg transmission (14).

Solid information on the epizootiology and transmission of AE was slow to surface, largely because of the lack of knowledge of the immunology of the infection and the consequent unknown susceptibility status of chickens used in experimental infection and transmission trials. Also, many experiments were done with a virus strain (later called the Van Roekel strain) that was passaged many times by serial intracerebral inoculation (51) and is now known to induce a pattern of pathogenesis different from that induced by field strains (14,45).

As already noted, Jones (28) suspected that the virus was transmitted through the egg because of the very early posthatch appearance of clinical disease in affected flocks. However, she was unable to demonstrate virus in embryos derived from flocks that had previously yielded infected chicks. Three laboratories took up the challenge to identify methods of transmission and to effect experimental transmission of the disease by other than intracerebral inoculation of young chickens. Peter Olitsky and others at The Rockefeller Institute in New York carried out some transmission experiments in the late 1930s. However, most of the early studies on transmission were conducted by Henry Van Roekel and colleagues at the University of Massachusetts in Amherst and by Erwin Jungherr's group at the University of Connecticut in Storrs. Van Roekel's group cited circumstantial evidence of eggborne infection from field cases and observed neonatal clinical disease in chicks hatched from eggs inoculated with AE virus at various times of incubation (52,53). They correctly postulated that the source of infection in affected flocks was from chicks hatched from infected eggs. Jungherr and Minard (30) confirmed Van Roekel's findings, but evidence from Jungherr's and Olitsky's laboratories (30,32) suggested that the virus was not multiplying, but rather was only surviving, during the incubation period. In any case, none of the research groups had reproduced the complete cycle from infected hen to infected chick. Later efforts to adapt the virus to chicken embryos by "zig-zag" passage of virus back and forth between embryonating eggs and young chicks also failed to demonstrate growth of the virus in the former.

Incubator transmission was strongly suspected, and experimental direct transmission by contact between infected and normal chicks was reported by Van Roekel, Bullis and Clarke in 1939 (52). Both the Connecticut and Massachusetts groups cited field observations that suggested contact spread (30,52). Unfortunately, the natural route of horizontal infection was not uncovered. Neither Jones (29) nor Olitsky (36) had been able to transmit the disease by the oral route. Although Van Roekel et al. (52) claimed transmission by intranasal instillation of virus, this route was not confirmed by Olitsky (36). The only successful routes of infection were those requiring injection of virus, i.e., intramuscular, intraperitoneal, intradermal, intrasciatic, intraocular, and intracerebral (29,30,36,51), hardly offering an explanation of the natural means of spread. Also, routes other than intracerebral were not always successful in all laboratories. An observation reported by Jungherr and Minard in 1942 (30), but unconfirmed at that time, was prophetic: they found that feces from affected flocks, and to a lesser extent from normal flocks, caused histologic lesions (but no clinical signs) of AE when inoculated into chicks.

Little was known about immunity in AE for many years. In 1939, Olitsky (36) had demonstrated the presence of neutralizing antibodies in the serum of recovered chickens, a finding confirmed by Jungherr and Minard shortly thereafter (30) and also by Feibel *et al.* in 1952 (21). Furthermore, although it was known that only young chicks showed signs of the disease, and that the postulated egg transmission would have to come from apparently normal breeders free of any clinical disease, the concept of age resistance did not surface at that time. A major difficulty was the absence of good test systems for assaying virus and antibodies. For about a decade after the early 1940s, little appeared in the literature other than numerous reports of the disease being identified in additional areas or species. Also, by that time many of the earlier players discontinued studies on AE.

DEVELOPMENT OF METHODS AND TESTS

It was not until the 1950s that significant breakthroughs in methodology plus a few key observations began to open the door to eventual understanding of the epizootiology and immunology of the disease. This, of course, was the key to eventual control methods. Although most of the people involved in the initial definition of the disease had dropped investigations on AE, Erwin Jungherr in Connecticut was an exception. Beginning with Frederick Feibel in the early 1950s, a succession of graduate students in Jungherr's laboratory took on AE as the subject of their thesis research and, together with other colleagues in the department, they made remarkable progress in methodology that was critical to subsequent work there and elsewhere. Their work stimulated others to join the fray, including Bruce Calnek, the author of this review. As an aside, it might be mentioned that

when Calnek joined the faculty at the University of Massachusetts as a young (25 yr old) scientist starting his first job in 1957, he wanted to work on mycoplasma infections, but that disease was "off limits" because Henry Van Roekel was working on it. AE was among the alternate possibilities but Kenneth Bullis, the Department Chairman, after first checking with Van Roekel, informed Calnek that because Van Roekel "might want to work on it again sometime in the future," it, too, was a nonviable choice. Ownership of diseases was a new concept to Calnek, and only after some strong arguments did Van Roekel "relent," and Calnek was finally given permission to work on AE.

The series of studies that reopened and greatly expanded the field of AE investigations began with a report by Feibel *et al.* (21) who, like others before, tried but failed to infect chicken embryos by a variety of routes including the allantoic and amnionic cavities, the chorioallantoic membrane and the yolk sac. However, they were able to infect young chickens inoculated intraocularly (i.e., directly into the eyeball), and adult chickens were successfully infected by intraperitoneal injection of virus. In all trials, they had used the 135th passage of the Van Roekel strain of virus. Feibel (20) believed that the successful infection of adult birds could ". . . enlighten work along the line of detecting the suspected adult carrier birds," which could in turn ". . . help reduce the incidence of the disease." But the success of the intraocular route of infection was to have the greatest impact by serving as the stimulus for subsequent studies by Felix Sumner, Roy Luginbuhl, and Erwin Jungherr. They were able to "adapt" the Van Roekel strain of AE virus (150th chick passage) to embryos by this route for the inoculation of 9-to-11-day-old embryos (43). Over the course of 15 serial passages, with embryo brain and eye as the inoculum, consistent changes were observed in infected embryos, including sluggishness, paralysis, and death. This method of infection permitted the use of embryos in virus neutralization tests for AE antibodies for the first time. The virus was subsequently cultivated for 15 passages in the chorioallantoic cavity with similar results but with lower virus titers than seen following intraocular injections. Equally significant in impact was the finding that changing the source of embryos decreased the efficacy of infection, leading to the conclusion that failure to infect some embryos might be because of the presence of maternally derived antibodies. That hypothesis was proven in a second study by the same authors (44) in which they described an embryo-susceptibility test for assessing the AE immunity status of breeding flocks. On the basis of virus titrations by the intraocular route, they found that only 4 of 119 flocks produced embryos that were fully susceptible to AE virus. The significance of their findings is difficult to overstate; they not only explained why previous attempts to infect embryos had been unsuccessful (assuming that the embryos used came from resistant flocks), but they finally allowed studies on the pathogenesis of the disease that had been stymied for lack of suitable virus assay and serologic methods. They also pointed up the importance of serendipity in that, by chance, the breeder supply flock first employed by Sumner and his colleagues happened to be one of the few that could support the growth of AE virus. Indeed, the importance of having an AE-susceptible flock of breeder chickens was so great that it was the major motivation for the creation of the first specific-pathogen-free flock at the University of Connecticut (R. E. Luginbuhl, pers. comm., 1996).

It is important to define the term "adaptation" as it is applied to AE virus because it refers to changes in biologic properties that have a marked impact on the behavior of the virus in chickens and embryos (14). Natural field strains are enterotropic and are transmitted horizontally. Virus gains entry by the oral route and is shed in feces. Infection with this type of virus is relatively apathogenic in chickens more than 3 to 4 wk of age. It can replicate in chicken embryos but does not cause lesions. Infection of susceptible adult chickens induces a temporary drop in egg production and the virus is transmitted vertically. Young, immunologically immature chicks, infected either vertically or horizontally, develop neurologic signs, as do older chickens if they are infected by the intracerebral route.

A second pathotype, called adapted virus, evolves following repeated passage of the virus by intracerebral inoculation of infected brain material in chickens. Similarly, embryo adaptation may occur in some cases when the virus is serially passaged in susceptible chicken embryos (see below). Viruses which become adapt-

ed by either method, probably as the result of selection of mutants (35), appear to behave similarly. They are highly neurotropic and cause severe neurologic signs in chickens of any age following intracerebral inoculation. They also may cause severe neurologic signs in a proportion of chickens inoculated by other parenteral routes. However, they have lost their enterotropic properties and do not infect via the oral route except when very high doses are used, nor are they transmitted horizontally. When a virus is adapted, either by intracerebral passage or by passage in embryonating eggs, it acquires the distinctive property of pathogenicity for embryos. Infected embryos are paralyzed (limbs are rigid) and have a severe muscular dystrophy.

The presumption that the strain of virus used by the Connecticut workers (43) was "adapted" to grow in embryos following intraocular inoculation was probably in error; apparently the virus had already been modified sufficiently by the large number of intracerebral passages in young chicks made by Van Roekel. Calnek and his colleague, Hubert Jehnich, showed (12) that susceptible embryos developed typical lesions when inoculated as a first passage into the yolk sac with the 150th chick passage of Van Roekel strain AE virus, the same inoculum source used by the Connecticut workers. Field strains of AE virus did not require adaptation to grow in susceptible embryos but could, in some cases, become adapted to induce lesions. Frank Wills and Irwin ("Pinky") Moulthrop, in Salisbury, MD, used a field strain that had received only six serial intracerebral inoculation passages in chicks to inoculate known susceptible embryos via the yolk sac (58). Embryonic brain was used for 17 serial passages. Embryos examined during the first six passages contained virus but were normal in appearance. Beginning with the seventh passage and thereafter, embryos with encephalomalacia and muscular dystrophy characteristic of infection with the Van Roekel strain of virus (31) were observed. Following the example set by the Maryland workers, Calnek *et al.* (17) also propagated field strains of AE virus in susceptible embryos without evidence of lesions, although occasionally an isolate would "adapt" after several serial passages (17). The proven ability of AE virus to grow in embryos without undergoing adaptation, i.e., without inducing characteristic lesions, turned out to be critical in the ultimate development of live virus vaccines against AE (see later).

FINAL DEFINITION OF THE EPIZOOTIOLOGY AND PATHOGENESIS OF AE VIRUS INFECTION

Another interesting finding by the Summer group was that one of the flocks they surveyed by the embryo-susceptibility test (44) produced eggs that became increasingly resistant during the course of the study and this correlated, time-wise, with a temporary drop in egg production in the flock. This finding was particularly interesting in view of an observation 2 yr earlier by L. Taylor, D. C. Lowry, and L. G. Raggi at the University of California, who reported a brief drop in egg production that was followed by the appearance of AE in chicks hatched from eggs laid during the drop (49). Although an effect of AE on egg production had not been observed by earlier workers, the type of temporary drop noted by the California group and subsequently confirmed by the Connecticut workers (44) was later found to be consistent enough to allow astute observers to often predict when an AE "break" occurred in breeding flocks. Levine (33) considered the observation by Taylor *et al.* to be "the first step in clarifying the epizootiology of the disease."

Use of the yolk sac inoculation route with the Van Roekel egg-adapted strain permitted simple and relatively rapid virus neutralization tests for AE virus antibodies (12). This tool, coupled with the availability of known susceptible eggs and chickens, finally permitted definitive studies on epizootiology and pathogenesis of the infection. Equally important was the ability to work with virus strains that had not been altered by a large number of intracerebral passages in chicks, but rather could be studied as natural, unmodified field isolates. This was a critical point as it turned out.

Thus the stage was set for the investigations of epizootiology carried out in the late 1950s by Calnek and his colleagues at the University of Massachusetts. After a series of experiments exploiting the newly developed yolk-sac virus neutralization test for antibodies (12) to evaluate responses to various vaccination procedures (13) (see later), they decided to concentrate on studies with freshly isolated field virus. Until that time, aside from the early experi-

mental work by Jones and others in the 1930s (when they were undoubtedly working with immune recipients), virtually all transmission studies had been carried out using the high passage Van Roekel strain. Without realizing the importance of the decision at that time, the switch to fresh field strains was clearly a fortuitous choice because adapted strains of AE virus were found to have lost their enterotropic properties while retaining their neurotropic character and thus are poorly able to infect chickens by natural routes (14). Calnek, along with Patricia Taylor and Martin Sevoian, published their findings on transmission in 1960 (17). They obtained definitive evidence that AE virus causes an enteric infection in which virus is spread by the fecal–oral route, nicely substantiating the earlier discovery by Jungherr and Minard (30) that feces from infected birds carried the virus. Furthermore, they finally experimentally completed the cycle of infection from exposure of hens to the AE virus through to the appearance of the disease in progeny chicks. Also, they obtained solid experimental evidence that contact transmission is readily effected in the incubator as well as the brooder. They obtained results in agreement with the observations made by Taylor *et al.* (49) in that both egg production and hatchability in the experimental infection were adversely affected for a period of a few days coincident with the period of active enteric infection and fecal shedding, all of which subsided with the appearance of serum antibodies. The lack of horizontal transmission to chicks hatched from eggs laid after antibody production commenced in the dams explained many of the early field observations on the pattern of disease appearance in various hatches. Furthermore, the finding that chicks infected by egg transmission developed signs within the first week posthatch whereas those infected by contact exposure did not show clinical disease until after 11 or more days posthatch provided a basis for the early observations that some chicks developed the disease shortly after hatching whereas the majority did so after 2–3 wk. Although most affected chicks succumb, a few with neurologic signs may survive. Some of these may later become blind due to cataracts (8,38,59).

The pathogenesis of the infection was to be worked out shortly after dependable experimental transmission methods were reported. Calnek *et al.* (17) had shown that following ingestion of AE virus, replication in the alimentary tract ensued with virus excretion in the feces during the second week postexposure, ceasing coincident with the development of antibodies. But it was an elegant study by Norman Cheville at the National Animal Disease Laboratory in Ames, IA that clarified the issue (19). He learned that thymectomy was without effect on the disease, but bursectomy totally eliminated the age resistance that normally prevented clinical disease in chicks infected after 3–5 wk of age. A series of experiments by Harvey Westbury and Barry Sinkovic at the University of Sydney in Australia (54, 55, 56, 57) confirmed and extended Cheville's observations; they further determined that there was a relationship between the development of antibodies and the ability of the virus infection to proceed from a viremia to a progressive CNS infection. Clinical disease resulted from CNS infection only if not terminated early enough by the immune response. They also clarified various aspects of the role for maternal antibodies in protecting progeny from immune dams when exposed to virus within the first few weeks of life.

DEVELOPMENT OF CONTROL PROCEDURES

Unfortunately, while the scientists were fiddling, the poultry breeder industry was burning; the industry could not wait for the orderly development of methods and information that would ultimately lead to control of AE. Part of the problem was that from the beginning it was considered probable, if not almost certain, that this was an egg-borne disease. That shifted the focus to the hatchery as the responsible unit. According to Feibel (20), a court case in Connecticut set a precedent in 1951 by allowing a poultry producer to deduct the cost of chicks lost due to AE from the bill for chicks purchased from the hatchery. Not only was this a direct economic hazard to the hatchery owner, but it also had indirect effects by causing the purchasers of chicks to be wary of hatcheries that had given them a problem with AE in previous lots. P. Philip Levine at Cornell University liked to tell a story (P. P. Levine, pers. comm., 1954) about an incident involving Monroe Babcock when he was just starting in the breed-

ing and hatchery business in Ithaca, NY. Babcock had just learned that a particular hatch was infected with AE following a call from Levine who had made the diagnosis. So Babcock kept his eyes open for angry customers who also received chicks from the same hatch and were therefore expected to return demanding refunds. When he saw one such customer driving up, he went out with cash ready in hand and passed it over without any words yet spoken. According to the story, the customer was so impressed that he immediately returned the money with an order for replacement chicks.

The disease had become sufficiently prevalent by the late 1940s and early 1950s that large breeding organizations were very concerned. Kimber Farms in Niles, CA, knew where their AE problem originated. According to Walter Hughes (W. F. Hughes, pers. comm., 1996) they introduced one group of eggs from New England in 1941, and in 1942 they saw their first case of AE. Kermit Schaaf was the veterinarian at Kimber Farms at that time, but he soon left for a period of 5 yr. He returned in 1949, only to be confronted with a serious outbreak of AE, and was immediately assigned the task of determining what could be done. Kimber Farms was not the only company so afflicted with this new problem. Donald Zander was hired as the veterinarian for the Heisdorf and Nelson (H&N) poultry breeding organization in Kirkland, and later Redmond, WA, in 1955. When he was recently asked if H&N had a problem with AE at that time, he replied (D. V. Zander, pers. comm., 1996) "Yes, they had AE coming out of their ears!" and his first assignment was to tackle the problem. Similarly, according to John Taylor (J. R. E. Taylor, pers. comm., 1997), when he joined DeKalb Agricultural Research in DeKalb, IL, in the mid 1950s, "The three problems I soon learned to be paramount were AE, chronic respiratory disease and Newcastle disease. The AE problem throughout our agent-hatchery spring hatches was enormous! I recall 142 counted outbreaks—separate and distinct, and obviously thousands of dollars of loss and adjustments."

It is not difficult to understand that the urgency associated with AE outbreaks in the poultry industry demanded action, and two control approaches were soon developed for use in the field. One, of course, was the vaccination first implemented by Kimber Farms and later adopted by H&N. The other, initiated a few years later by DeKalb, was an embryo-testing program to identify nonimmune breeder flocks that could be a risk for future breaks. The latter approach was then coupled with a vaccination program applied to the antibody-negative flocks. Although the Kimber program preceded the DeKalb program, the latter is covered first in this review in order to permit all of the vaccines, including that used by DeKalb, to be discussed together.

Embryo-susceptibility tests to select resistant breeder flocks. The embryo-susceptibility test developed at the University of Connecticut and reported in 1957 (43,44) was to have an almost immediate impact in the field. John Taylor and Enos Schelling at the DeKalb Agricultural Association adopted a modification of the test, described earlier by Calnek *et al.* (17) in which a single dose of the Van Roekel strain of AE virus was inoculated via the yolk-sac of embryos. With this approach, they conducted a survey of over 2000 flocks from all across the United States and Canada (48). Nearly 60% of the flocks were found to be already positive for AE antibodies at 5 mo of age, and all but 4% were positive by 13–18 mo of age. They were quick to suggest that use of this test to select breeder flocks would help avoid the problem of egg transmission and, thus, clinical disease in the progeny. They were even quicker to embark on an enormously ambitious program in which they tested 10 fertile eggs from every "agent-hatchery" pullet flock in the DeKalb organization just as the flocks were starting in production (J. R. E. Taylor, pers. comm., 1997). The program involved hundreds of supply flocks ranging from 250 birds to 5000 birds scattered throughout the 48 states in the U.S.A. and various Canadian provinces. The owners of the agent-hatcheries were prohibited from setting eggs from any untested flock or from a flock that tested negative for AE antibodies until it turned positive on a subsequent test. Taylor recently recalled (J. R. E. Taylor, pers. comm., 1997) that the cooperation of agent-hatcheries was high (probably because DeKalb informed them that the parent company would not share in any "adjustments" if they had a problem with AE), and the "results were fabulous. . ...our problems went to zero, not a single case, and to this day I have difficulty believing it." Use of hatching eggs

rather than blood samples for immunity testing saved considerable expense and made it easier for agent-hatcheries to participate in the program. To the knowledge of the author of this review, this was the only application of this procedure in the commercial poultry industry; probably it would have been attractive to others had not vaccination procedures already been described by the Kimber organization (see later). On the other hand, unlike the Kimber vaccination scheme, this program did not address the problem of control itself.

The major difficulty with the DeKalb approach was that although 75–80% of the flocks were positive on the first test, the differential value of hatching eggs versus those marketed for consumption approached one dollar per dozen (J. R. E. Taylor, pers. comm., 1997) and so owners of antibody-negative flocks were losing money as well as being short of hatching eggs. AE is a particular problem for flocks maintained in modern housing with the best isolation so it was seen almost as a severe economic penalty for doing a superior job. This quickly prompted John Taylor to implement a vaccination program to assure rapid serologic conversion of the flocks found negative with the embryo-susceptibility test (46,47), (J. R. E. Taylor, pers. comm., 1997) (see later).

Vaccination programs. Early studies showed that an immune response to AE virus infection could be detected in convalescent chickens. In 1939, Olitsky (36) demonstrated virus neutralizing antibodies in serum of chicks that survived inoculation with the Van Roekel strain of virus, and Jungherr and Minard (30) confirmed the point in 1942. Yet, it was not until nearly two decades later that suitable virus neutralization tests were described by Sumner *et al.* (43) and Calnek and Jehnich (12). And, although such antibodies could be related to resistance of embryos and thus signal that a breeder flock had been exposed (44,48), it was not until 1960 that definitive experimental evidence of a protective effect of the antibodies in newly hatched chicks was reported by Calnek *et al.* (18).

The move toward vaccination as a control method did not wait for the orderly development of experimental data. As mentioned above, the disease had become a serious problem for poultry breeders by the 1940s. D. V. Zander (pers. comm., 1996) notes that veterinarians were becoming involved more and more with both broiler and layer breeding companies at about that time, and he speculates that they were actually part of the problem with AE. Because of the better sanitation and isolation they demanded, there were more breeder flocks that were still free of infection when they entered the production cycle. W. F. Hughes (pers. comm., 1996) noted that this improvement in sanitation and isolation was probably due in large part to cleanup attempts instituted by breeding organizations for the control of *Salmonella pullorum* infections. In any case, the veterinarians were expected to find a solution to the problem.

Vaccination was obviously a choice approach. The causative agent was known to be a virus and to induce antibodies. Unfortunately, there had been practically no reported attempts to immunize birds. The only suggestion that immunization might be a viable option came from a very limited experiment done by Jungherr and Minard in the early 1940s (30). They had been testing various noncerebral inoculation routes and observed that the Van Roekel strain failed to provide any "takes" except for the intravenous route (no details provided). They then challenged all remaining groups by intracerebral inoculation and found that the only birds to survive were those initially exposed by the intraperitoneal route. They duly noted that "The immunologic significance of [this] observation requires further scrutinizing." Either they failed to follow up on the point or they were unable to repeat the experiment for there was no subsequent published information from that group. Jungherr was quoted in 1955 (1) as saying "The time seems right to make a concerted attack on this [AE] problem and set up a pilot experiment to prove the feasibility of either eradication, vaccination, or both, under controlled conditions. Such an experiment could definitely point the way and eventually enable the poultry industry to control the disease."

The Kimber vaccination program. Thus, it was essentially without precedent or any real clues from prior reports that Kermit Schaaf at Kimber Farms in California embarked on his groundbreaking vaccination attempts in the early 1950s. Walter Hughes, who worked for many years at Kimber Farms, recalled (pers. comm., 1996) that when Kermit Schaaf first

became involved with AE in 1949, he used intracranial inoculation in titration tests to determine the infectivity of his virus, and from that, "... immediately, of course, came the vaccine production which was started in 1950."

Schaaf and his colleague, Welford ("Will") Lamoreaux, published their landmark paper entitled "Control of avian encephalomyelitis by vaccination" in the October, 1955 issue of the *American Journal of Veterinary Research* (41). This article contained a description of the 1949 AE break that involved progeny of young pullets but not those of older hens even though both groups of breeders were housed together with equal opportunity for virus exposure. They correctly concluded that the old hens were "protecting their chicks from the disease." Recognizing that the resistance probably resulted from immunity acquired from a previous unrecognized exposure, they decided to attempt deliberate exposure of prospective breeding stock to AE before they reached sexual maturity. In October 1950, with a brain suspension of virus propagated by intracerebral inoculation of young chicks, they cautiously administered their "vaccine" to about 2% of the pullets of one prospective breeding flock. The delivery system was a two-pronged vaccinating needle that was dipped into the brain suspension and then stabbed through the wing web. In subsequent years, all chicks were vaccinated in the same manner at about 18–20 wk of age. Eventually they combined the AE virus with Newcastle disease and fowl pox vaccines. A small number (average about 1%) of the vaccinated birds developed clinical signs. Schaaf later admitted (pers. comm., 1960) to the author of this review that the incidence of clinical disease in vaccinated birds might have been as low as it was only because many of the flocks probably already had been exposed to AE virus before they were inoculated. The success of their vaccination program was remarkable. During the first 6 yr in which they immunized prospective breeding stock, and reimmunized old hens at the beginning of their annual forced molt, over one million doses of live AE virus were administered without any major outbreak of the disease in approximately 30 million progeny chicks.

Alternate vaccination routes. Later, Schaaf experimented with alternate dosages and routes of delivery of his vaccine (39). By that time, the vaccine virus had received 35 serial passages by intracerebral inoculation of young chicks. He compared wing-web (WW) inoculation with intramuscular (IM) and *per os* administration and found that although IM inoculation gave superior protection against intracerebral challenge, it induced a higher incidence of clinical disease by itself in vaccinated chicks. Oral administration offered some protection against challenge, particularly with the highest dose tested, i.e., over one million chick lethal doses–50%, and birds exposed by contact with vaccinated penmates showed some evidence of immunity as well.

At about the same time that Schaaf did his alternate route studies, Calnek and Jehnich (13) were also experimenting with immunization procedures with the high-passage Van Roekel strain of AE virus. In their case, both serologic response and resistance to challenge were measured after administration of virus by the IM, WW, spray, drinking water, and *per os* routes. Their results confirmed and extended those of Schaaf by showing that IM or WW doses of 15,000 or more embryo infective doses–50% (EID_{50}) were required for a strong serologic response and that the postvaccinal signs of AE were more prevalent in IM-inoculated than in WW-inoculated chickens. Spray administration was quite pathogenic for recipients but was only poorly immunogenic. Oral administration, either through the drinking water or by direct pipetting into the mouth or crop, was the only vaccination route that did not induce clinical signs in the recipients, but, as was the case in Schaaf's experiments, it was protective only when very high doses (over one million EID_{50}) were used. Therefore, from both practical experience (41) and experimental studies (13,39) parenteral administration (vaccination) with AE virus propagated by serial intracerebral passage appeared to be immunogenic but was not without some risks because of the pathogenicity of the virus.

To the great credit of the organization, the Kimber experience was generously shared with both competitors and noncommercial scientists. An announcement from the company, which was distributed by way of a hatchery newsletter (Kimber Chick News) and reported in a poultry journal (2), stated: "On November 12, 1955, the Board of Directors of Kimber Farms, Inc. voted unanimously to make samples of the

seed virus immediately available at no cost to any experiment station, biological laboratory or qualified veterinarian, for further study, tests, and subsequent distribution, subject only to any legal restrictions, federal or state, which affect the use or movement of vaccines." One wonders if such open generosity will ever be seen again in this era of fierce competition, secrecy, patents, etc. At least one other large breeding organization was quick to follow the lead of Kimber Farms. Donald Zander, confronted with the need to establish an AE control program at the H&N organization in Washington, indicated that Schaaf and Lamoreaux at Kimber Farms were "pretty free with information . . . we isolated our own virus from the field, but the technique we used was theirs" (D. V. Zander, pers. comm., 1996). Both Kimber Farms and H&N continued to produce and use their own chick brain vaccine even after a commercial vaccine was available, but to the knowledge of Walter Hughes (pers. comm., 1996), Donald Zander (pers. comm., 1996), Roy Luginbuhl (pers. comm., 1996), and Bruce Calnek, no other commercial company ever took advantage of the Kimber offer. At least one large company, DeKalb Agricultural Association, Inc., developed its own vaccine strategy (see below); others apparently simply held off until a commercial vaccine was available.

The DeKalb oral vaccination program. The experimental evidence (39) that the oral route might be safer and still protective was interesting, but Kimber Farms continued to use their field-tested (and trusted) method of WW administration. However, when John Taylor at the DeKalb Agricultural Association was confronted with the need to immunize susceptible young breeder flocks, he took advantage of drinking water or *per os* vaccination. He recently recalled (pers. comm., 1997) his first vaccination attempts: "I took the bull by the horns and gave some nearby flocks neat virus from embryos and stood well back. The method of exposure was really crude . . . I would shoot a few drops down the throat of about six birds, and for good measure pour the rest of the vial into a few drinking troughs . . . the flocks conveniently went into a dip in production . . . they then tested solidly positive." Taylor further related that he concurrently asked for and received permission for limited field trials from Dr. John Hejl, the Director of the United States Department of Agriculture (USDA) Biologics Division, but "fortunately, he did not define what 'limited trials' meant and I took off . . ." Taylor probably knew of Schaaf's experiments with oral vaccination (39) and he was most certainly aware of the studies being carried out in Calnek's laboratory (12) because a research grant awarded to Calnek from DeKalb had allowed him access to their results before publication. This knowledge probably helped allay any fears he may have had about trying that approach on a large scale. Regardless, he must be given full credit for putting the method to the test in the field. He subsequently reported (46) that he had initiated large-scale vaccination trials in the field through the cooperation of state and federal authorities, and that he would be collecting data on efficacy, effect on egg production, spread, and egg transmission, but no further reports ensued. However, according to Taylor (pers. comm., 1997), essentially all flocks that tested negative for antibodies by the embryo-susceptibility test were vaccinated and there were ". . . very few misses—probably not more than five during the entire time."

A "red herring" cell culture–adapted AE virus. In 1959, an apparent major breakthrough was announced by Jen Hwang, Roy Luginbuhl, and Erwin Jungherr from the University of Connecticut (27). They reported the successful adaptation of AE virus to chick kidney tissue culture and soon thereafter described a virus neutralization test for AE antibodies (25). There was widespread publicity of this finding as the "answer" to the AE problem and newspaper reports indicated that the Connecticut workers were very close to field trials to test the virus as a vaccine (3). Unfortunately, they did all of their serologic work with the tissue culture–adapted virus as the antigen. Their virus was subsequently found to be unrelated to the Van Roekel strain of AE virus (B. W. Calnek, unpubl. obs., 1960), and they were forced to admit that, indeed, a mistake had been made and that the virus was actually a contaminant avian adenovirus (26). AE virus was eventually grown successfully in several types of cell cultures (14,45) but not for vaccine production.

Nonadapted strains of AE virus for oral vaccination. The high doses of virus that appeared to be necessary for oral vaccination made the procedure less than ideal. On the other hand,

the study on epizootiology conducted by Calnek *et al.* (17) suggested that nonadapted field strains of virus might be better candidates for an oral vaccine because 1) they easily infected birds inoculated *per os*, 2) they did not induce clinical disease in growing or mature birds and 3) they spread by contact. One of the field isolates used in the epizootiology studies was selected for further testing as a vaccine. The strain, called #1143, was isolated on December 24, 1957, from AE-afflicted chicks obtained from Harco Orchards, a poultry breeder in eastern Massachusetts. The virus was tested for safety and efficacy as an oral vaccine after the sixth embryo passage (yolk sac route) and was compared with three embryo-adapted strains of AE virus: 1) the Van Roekel strain, 2) isolate #86, a field strain that became embryo adapted after eight embryo passages, and 3) isolate TAY, the DeKalb vaccine strain that was found to be completely embryo adapted upon receipt from John Taylor (18). The results with strain #1143 were extremely encouraging and formed the basis for the eventual development of commercial vaccines that are still in use throughout the world. Whereas all three egg-adapted strains were only weakly immunogenic and failed to be shed in the feces following oral administration, even with doses of 1–1.5 million EID_{50}, very low doses of #1143 virus induced substantial serologic responses and were shed in the feces. Field trials involving oral vaccination of six flocks of mature chickens and 12 flocks of growing chickens demonstrated that the #1143 strain was consistently efficacious in terms of inducing serologic responses and resistance to intracerebral challenge and the infection mimicked the natural infection by spreading to contact controls and causing a temporary slump in egg production without any neurologic signs (18). Administration of as few as 10 minimal infective doses per bird was effective by either drinking water or *per os* route. With the *per os* route of administration, as few as 1% of the birds could be inoculated with the knowledge that the virus would spread to penmates. Following these studies, the disadvantages previously associated with oral vaccination (high dose requirement, lack of spread) appeared to be solved, and thus a practical vaccine suitable for mass administration was in hand. Calnek soon received permission from state authorities to produce the vaccine for intrastate use. The virus was subsequently made available for administration to all breeding organizations within Massachusetts until a licensed vaccine manufacturer could be persuaded to produce the vaccine.

Other vaccines and methods of administration. Strain #1143 and similar unadapted, embryo-propagated strains have been used as AE vaccines throughout the world since the early to mid 1960s. However, there was a perceived need for an inactivated product that could be used to protect flocks already in production or in areas where there was concern about the spread of virus from vaccinated flocks to nearby nonimmune breeders. Several reports of efficacious AE vaccines inactivated with beta-propiolactone were published (9,16,34,40). These vaccines never saw widespread use because of their greater production costs and the need for at least two labor-intensive inoculations but have nevertheless served a useful purpose. Slower and less efficient spread of vaccine virus among birds in cages compared with those raised on the floor (22,42) led to the use of spray administration of strain #1143 in some cases (22), and Glisson (23) noted that many commercial breeder and layer replacements are now routinely vaccinated by the WW route. Parenteral administration of the milder vaccines is probably safe providing the virus has not been allowed to become embryo adapted during production. The USDA requires that vaccine virus be no further removed than five passages from brain passage, and that immunogenicity testing be conducted on the highest passage reflected in the final vaccine. Even so, Glisson (23) reported that the level of embryo adaptation, varies among manufacturers. Only one of five commercial vaccines tested had no evidence of embryo adaptation whereas the other four induced lesions of muscular dystrophy in 12%–100% of inoculated embryos. He concluded that the partially or completely embryo-adapted vaccines were probably responsible for the 1%–4% incidence of clinical AE seen in some vaccinated flocks, not unlike the situation reported in the 1950s with the Kimber Farms vaccine. A similar speculation about inadvertent egg adaptation of AE virus by vaccine manufacturers was offered in a case report of clinical encephalitis in vaccinated broiler breeder pullets (24).

LICENSING OF AE VACCINE

Although three major poultry breeders in the U.S.A. (Kimber Farms, H&N Nelson, and DeKalb) had embarked on ambitious vaccination "trial" programs with the blessing of appropriate state and federal authorities, the situation was far from resolved as far as long-term control was concerned. Indeed, the conclusion of the story of AE control is one of delay and some frustration. In 1962, Gerald Peacock, from the USDA, presented a paper at the Northeast Conference on Avian Diseases in which he summarized regulatory activities concerned with the development of AE vaccine (37). He indicated that the Animal Inspection and Quarantine Division had been approached about a license for AE vaccine as early as 1956, but prospective license applicants became discouraged by the amount of work necessary to determine the efficacy of experimental products, particularly at a time when the mode of natural transmission and true incidence of the disease were still unknown. The Division gave approval (to the above breeding organizations) for limited field studies, but according to Peacock, those trials were unsatisfactory ". . . because of failure to provide unvaccinated controls and to check immunity of vaccinates and their progeny by challenge or laboratory procedures." He further indicated that many requests for field trial permits were denied because they came from individuals who simply wanted to vaccinate their chickens but did not want to do a controlled trial, and presumably they either ran the risk of AE or found another source of vaccine.

By 1960, when the demand for AE vaccine was continuing to increase and information on both the incidence of infection (48) and the epizootiology (17) had been obtained, the government found itself in a dilemma. They needed to stop routine interstate shipment of unlicensed vaccine and find a way to have it replaced it with licensed product. In short, they wanted to revoke the permits for the "field trials" that had allowed Kimber Farms, H&N, DeKalb, and perhaps, others from continuing their wholesale use of "experimental vaccines."

To try to resolve the problem, John Hejl, the Director of the Division of Veterinary Biologics in the USDA, requested a meeting of industry and university personnel. John Taylor, Kermit Schaaf, and Donald Zander, the individuals who were conducting the so-called "limited field trials" with their own AE virus preparations, represented commercial poultry breeder organizations. Roy Luginbuhl from the University of Connecticut and Bruce Calnek from the University of Massachusetts were asked to participate as individuals from academia with a strong interest in AE control. The meeting took place in 1961 in Washington and it was clear from the start that Hejl was unhappy with the nearly total lack of information coming from the breeders who had been "testing" their vaccines for a period of several years (J. Hejl, pers. comm., 1961). In effect, he threatened to recall the permits if things were not turned around so that useful data were accumulated. He had a point. John Taylor recalls that ". . . reporting to Dr. Hejl was somewhat tongue-in-cheek, though we got a little more sophisticated as time went on. I listed every trial and the results in the hope of impressing him with sheer numbers . . . but he kept asking for unvaccinated controls on the vaccinated farms . . .." Of course, Taylor and the others had to point out that unvaccinated controls were difficult to keep because the virus spread rapidly and that carrying out well-controlled field experiments with this virus was not easy. The three industry people were delighted that Luginbuhl and Calnek were involved in discussions. They believed (J. R. E. Taylor, pers. comm., 1997; D. V. Zander, pers. comm., 1996), probably correctly, that Hejl perceived a credibility gap with the industry people who clearly had an axe to grind. In any case, several hours of very serious, and sometimes heated, discussions took place over a 2-day period in John Hejl's office. The biggest hurdle was to convince him that use of a live virus to vaccinate prospective breeder flocks would not increase the infection rate in nonvaccinated flocks to which it might spread. The clincher probably was the DeKalb study (48) showing that practically all flocks eventually became infected anyway, coupled with the fact that the vaccine developed by Calnek *et al.* (18) caused infections essentially identical to natural infections. The plea on the part of all of the participants of the meeting was for a program that essentially would only guarantee the timing of infection (before production). Hejl finally agreed, to the relief of all present. Peacock later noted (37) that ". . . efforts then

were directed toward stimulating interest by a biologics manufacturer [in] further evaluating live virus vaccine propagated in chicken embryos. . .," and that "Prospective licensees agreed to withdraw pending license applications for chicken brain–propagated live virus vaccine."

At about this time, a committee of the Northeast Conference on Avian Diseases developed and published a tentative program for the control of AE (15). They considered two general approaches: hatching only from immune flocks and immunization. The former was believed to be less advantageous than the latter because it did nothing to control the disease itself and was useful only for hatcheries with enough supply flocks to allow selection. Three potential immunization programs were discussed. WW or IM with live virus was thought to have the major disadvantages of having to handle each bird individually and the possibility of a rather severe postvaccination reaction in some cases. The second program, use of beta-propiolactone–inactivated vaccine, was faulted for being costly as well as requiring handling of individual birds. The favored approach was the third potential program, i.e., the use of a live virus for oral administration between 8 and 20 wk of age, in conjunction with the use of inactivated vaccine for susceptible flocks already in production or in areas where the disease is not enzootic. The committee recommendation, therefore, fit in very nicely with the objective of the Biologics Division to encourage licensure of the live virus vaccine for oral administration.

The next step, obviously, was to get a commercial vaccine manufacturer interested in licensing a product that required a good deal of field testing but would be produced for use in breeder-replacement flocks constituting a relatively small market. John Taylor had a strong interest in a licensed live vaccine for oral administration because of the large-scale testing/vaccination program that he had instituted for DeKalb. He recalled (J. R. E. Taylor, pers. comm., 1997) that DeKalb did not want to get in the vaccine business, and so he approached several vaccine companies, notably Abbott Laboratories, Vineland Laboratories, and Salsbury Laboratories. The first two turned him down for economic reasons (small market), but Salsbury Laboratories, in Charles City, IA, agreed to go ahead "for the good of the industry."

Gerald Peacock (37) referred to a cooperative effort by "a large breeding establishment" (DeKalb) and "a manufacturer of poultry biologics" (Salsbury). He noted that a number of setbacks had occurred in their field testing program, one of which may have been "excessive embryo passages." This is probable given Calnek's finding that the strain of vaccine virus used by Taylor had become fully embryo adapted and was no longer able to infect efficiently by oral administration (18). Taylor, who had been kept fully informed of the work on an oral vaccine at the University of Massachusetts, suggested to Calnek that he make strain #1143 available to Salsbury Laboratories. Toward that end, they both visited with Drs. Oliver Peterson and Peter Matishek at the Salsbury vaccine production plant in Charles City, IA, and both the virus strain and Calnek's vaccine-preparation technology (prepublication) were given to the company (with great relief knowing that *someone* would produce the vaccine and seek licensure). John Taylor and the DeKalb organization cooperated fully with Salsbury Laboratories by continuing to conduct necessary field trials. This time the trials worked well and in July 1961, at the American Poultry Congress in Minneapolis, Peterson and Taylor were able to announce that a license application for the vaccine was to be made soon (4). On March 7, 1962, Avian Encephalomyelitis Vaccine, Live Virus, Chick Embryo Origin was licensed by the federal authorities for interstate distribution (37).

Without question, the use of vaccination as a practical control approach for AE can clearly be credited to Kermit Schaaf, who showed the way and provided the stimulus for further work on immunization. However, it was the elucidation of the epizootiology and pathogenesis of the infection by Bruce Calnek and his colleagues that led to the development of the first practical embryo-propagated vaccine. Finally, had it not been for John Taylor's perseverance and ingenuity, the licensing of the "Calnek strain" as a commercially available vaccine for the control of AE might have had a much longer gestation period.

SUBSEQUENT WORK

Thus, the work that led to licensing of AE vaccine was essentially completed by 1962. The

original commercial vaccine and similar vaccines developed in other countries have stood the test of time, and it can be said that the disease problem was essentially solved when commercial vaccines were made available, aside from the occasional break due to vaccine errors (23,24). Since the introduction of commercial AE vaccine, manufacturers have refined vaccine production methods, utilized methods for stabilizing and freeze-drying virus, and provided new products in which AE vaccine is combined with other vaccines, for example fowl pox virus, for WW administration. The vaccine is often used in egg-producing flocks to eliminate AE virus-induced temporary egg-production slumps as well as in breeder-replacement flocks for the prevention of vertical transmission to progeny.

Despite the solution of the problem in the field, work continues, even to the present. The pathogenesis of infection, immunologic features of the disease, characteristics of the virus, and improved diagnostic methods and serologic tests all have been subjects of studies by many investigators (10,11,14,23,45).

LESSONS TO BE LEARNED

Usually, there are lessons to be learned from any scientific endeavor such as the one reported here. In the case of the research leading to the control of AE, two points are particularly prominent. First, is the need to work with specific-pathogen-free (SPF) chickens for all basic studies. That was, of course, not possible when the initial work on AE was carried out, but even now that SPF chickens are readily available, there continue to be reports of biomedical research conducted using birds of unknown disease and/or antibody status. This is particularly true for species other than chickens. Certainly, the solution to the AE problem was hindered to a very great extent by the lack of SPF birds. A second major lesson relates to the selection of a "prototype virus" not representative of that causing the problem. It was convenient to employ the Van Roekel strain of virus because it was characterized to some extent and because it had clear-cut pathogenicity under selected experimental conditions. Unfortunately, it was not at all representative of the naturally occurring virus associated with the field problem under investigation. Indeed, it was not until fresh field isolates were studied that any real progress was made toward understanding the transmission and pathogenesis of infection. An understanding of both transmission and pathogenesis is critical to finally achieving control. No doubt laboratory strains have much to offer, but it is important to remember that laboratory manipulation generally carries the great risk of selection pressure resulting in mutants that are no longer suitable for much of the research needed to develop control methods.

CONCLUSIONS

Over 30 yr transpired from the time that AE was first identified until the issue of control was finally laid to rest. Each of the chapters in the story was important: the early description of the disease itself along with some concepts of how it was transmitted; the development of a strain of virus (Van Roekel strain) with modified properties that turned out to be important in the first successful embryo infections and serologic tests; employment of embryo infection techniques leading to an understanding of immune status of flocks and development of testing methods; large-scale implementation of effective immunization schemes with crude infected brain suspensions as vaccines; recognition of the differences between adapted strains of virus and field strains, particularly in their neurotropic *vs* enterotropic properties; effective application of embryo susceptibility tests to determine the extent of field infection and to identify flocks that were safe to use as breeders; definition of the epizootiology and pathogenesis of the disease; development of live virus vaccines suitable for oral administration and inactivated vaccines for flocks in which live virus vaccination was contraindicated; and, finally, the field testing leading to licensing of commercial AE vaccines. The many roadblocks, particularly the huge one associated with the unidentified immune resistance of embryos and chicks used for most of the studies conducted during the first 25 yr of AE research, make it not surprising that progress was slow. The persistence of early workers and the innovativeness and cooperation of many later workers were necessary to ultimately solve the problem.

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