

Historical Article—

Fifty Years of Anticoccidial Vaccines for Poultry (1952–2002)

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This paper is dedicated to the memory of those pioneers whose work made possible the commercial success of anticoccidial vaccines and also to the late S. A. Edgar's grandson, Jake Giambrone, whose spirited determination to overcome his recent serious accident is a fine example to all.

SUMMARY. Although earlier investigators experimented with anticoccidial vaccines, the world's first commercially successful product was developed by Prof. S. A. Edgar of Auburn University, Auburn, AL. This product contained live, nonattenuated *Eimeria tenella* oocysts and was first marketed by Dorn and Mitchell, Inc., in 1952. Under the trade names of DM[®] Cecal Coccidiosis Vaccine, Coxine[®], NOBiCOX[®], and CocciVac[®], it went through several formulations containing various *Eimeria* species that parasitize chickens, and a further product containing turkey *Eimeria* species was also developed. After many product and company changes, one turkey and two chicken formulations of CocciVac[®] are still marketed worldwide by Schering-Plough Animal Health, Inc. Chicken and turkey formulations of Immucox[®], a similar type of vaccine, were developed by Dr. E.-H. Lee and first marketed in 1985 in Canada by Vetech Laboratories, Inc.

In 1974, Dr. T. K. Jeffers of Hess and Clark, Inc., Ashland, OH, published his discovery of precocious lines of coccidia, which facilitated the development of the first attenuated anticoccidial vaccine. For commercial reasons, Jeffers was unable to do this himself, but this first attenuated vaccine was designed by Dr. M. W. Shirley and colleagues at the Houghton Poultry Research Station (HPRS) in the United Kingdom. The vaccine was commercially developed under license in the United Kingdom by Glaxo Animal Health Ltd. and then Pitman-Moore, Inc., and launched in The Netherlands during 1989 under the trade name Paracox[®]. After further changes in company ownership, two formulations for chickens are now marketed worldwide by Schering-Plough Animal Health, Inc.

Attenuation of coccidia by embryo adaptation was reported in 1972 in the United Kingdom by Dr. P. L. Long, who originally worked at the HPRS and later became a professor at the University of Georgia, Athens, GA. An embryo-adapted line of *E. tenella* was included with precocious lines of other species in a series of three attenuated vaccines for chickens under the trade name Livacox[®], developed by Dr. P. Bedrník and launched in the Czech Republic in 1992 by Biopharm. The formulations of all other commercially available live anticoccidial vaccines for poultry are currently based upon the scientific principles established for the CocciVac[®], Paracox[®] or Livacox[®] vaccines.

**“Biographical research is a legitimate type of research for scientists—it puts flesh on the bones of knowledge”
(Norman D. Levine, 1973) (99)**

The year 2002 marks the golden jubilee of the world's first anticoccidial vaccine, CocciVac[®], which was the brainchild of Prof. Samuel Allen Edgar (1916–2000) of Auburn University,

Auburn, AL, USA (Fig. 1A). Therefore, now is an appropriate time to place on record some of the events that have led to the range of successful anticoccidial vaccines commercially available for poultry today. These vaccines probably already account for the greatest worldwide use of any kind of live vaccine against infection with a protozoan parasite (141). Several authors have addressed the history of re-

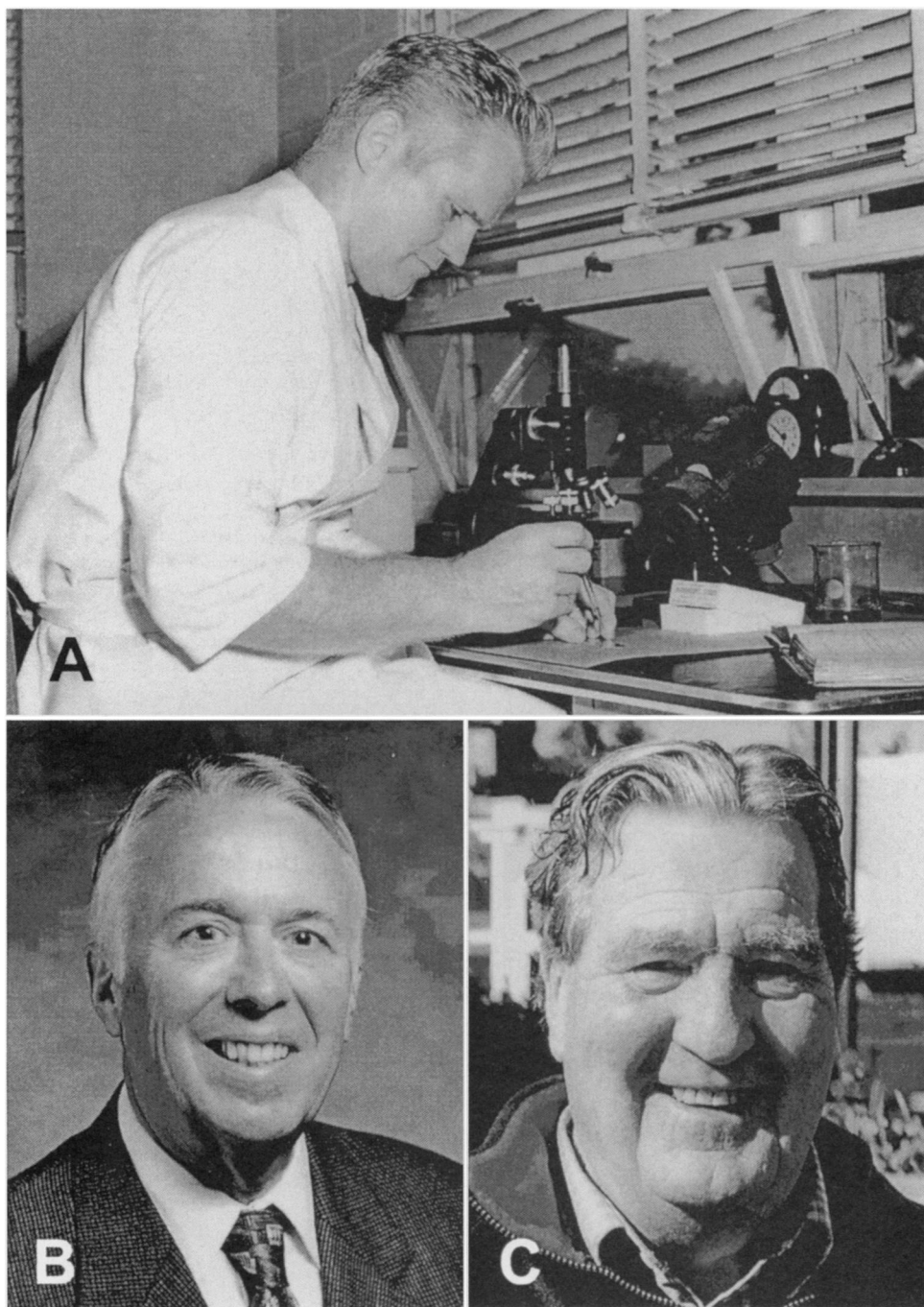


Fig. 1. (A) S. A. Edgar (when about 42 yr old), originator of the first anticoccidial vaccine, working in the Alabama Polytechnic Institute coccidiosis laboratory (see Fig. 3; photograph supplied by J. J. Giambrone). (B) T. K. Jeffers, discoverer of precocious attenuated strains of *Eimeria tenella* (photograph by Frank DiMeo of the Cornell University Photography Laboratory, 2001). (C) P. L. Long, discoverer of attenuation of *Eimeria tenella* by embryo adaptation (photograph by C. C. Wang, 2000).

search into poultry diseases in general (53,112) or coccidiosis in particular (51,99,125), but none of them have dealt with anticoccidial vaccines in any detail, if at all. My purpose here is to explore the scientific and commercial history of their development.

Three main lines of investigation into vaccine history have been followed: 1) the development of the world's first anticoccidial vaccine by Prof. S. A. Edgar; 2) the discovery by Dr. T. K. Jeffers of selection for precocity as a method of attenuation for vaccinal lines of coccidia; and 3) embryo adaptation of coccidia, an alternative method of vaccinal attenuation, demonstrated by Prof. P. L. Long. Various ramifications of these achievements have been traced, and particular emphasis has been placed on the individuals involved, their institutes or companies, and some scientific and commercial aspects of the resulting vaccines.

THE INTELLECTUAL CLIMATE OF AVIAN COCCIDIOSIS RESEARCH UP TO 1950

Any research on disease control must be underpinned by a clear understanding of the etiology and pathology of the disease, and this in turn is dependent upon accurate identification of the causative organism. Early taxonomic work on the coccidia was, as would be expected, somewhat muddled at first, but by the end of the 19th century, these parasites were recognized as a fairly distinct group in the old class Sporozoa of the phylum Protozoa; they now comprise a subclass of the phylum Apicomplexa (100).

During the last 20 yr of the 19th century, avian coccidia were placed in the genus *Coccidium*, which was described by R. Leuckart in 1879 (97) from its oocysts. A few years earlier, the genus *Eimeria* was defined by A. Schneider (133) mainly on the basis of the schizogonous stages. Therefore, until the complete life cycle of a coccidium was established by F. Schaudinn in 1900 (131), *Eimeria* was maintained as a genus separate from *Coccidium* (19,93,94,115). In 1902, C. W. Stiles (147) in the United States and M. Lühe (111) in Germany independently realized that the two names represented the same genus and that *Eimeria* was the valid name, being the senior synonym. Despite this, the name *Eimeria* did not displace the widely accepted usage of *Coccidium* for a considerable time afterward, perhaps because of the contin-

ued use of the term "coccidiosis" to describe the disease complex caused by these parasites (68,117). Even when the generic name *Eimeria* became well established, for some reason, "eimeriosis" never gained general acceptance for describing the disease.

Just as there had been confusion about the classification and nomenclature of the coccidia, for many years there had been considerable uncertainty about their pathogenic effects. Since the work in 1839 of T. G. Hake (67), who thought that oocysts were pus globules associated with liver carcinoma, there had been a steadily increasing stream of publications implicating the coccidia, or "psorosperms"—a term derived from yet another generic name, *Psorospermium* (99)—in the cause of cancers (see Hagenmüller [66] for bibliography). It was perhaps no coincidence, therefore, that Prof. Ernest Edward Tyzzer (1875–1965), regarded by many as the father of modern coccidiosis research, took up work on the coccidia some time before 1902 (112). Tyzzer was a George Fabyan professor of comparative pathology at Harvard University (125) and first and foremost a cancer researcher (80).

Having read Tyzzer's seminal works on fowl coccidiosis (151,154), one can appreciate the significance of his reminiscences in 1949: "When I started my investigations on avian infections somewhat over 30 years ago, there was little reliable information in regard to avian pathology . . . coccidiosis offers possibilities in genetic studies and also in questions which concern immunity" (153). Tyzzer ensured his place in the history of parasitology by helping unravel the confusion between coccidiosis and histomoniasis in turkeys (112), and he "unquestionably, more than any-one else, put the study of *Coccidia* on a critical basis with his early investigations of life cycles, biology and pathology of these parasites" (18).

However, Tyzzer did not have the field entirely to himself in those early days. J. R. Beach, of the California Agricultural Experimental Station, in 1917 distinguished coccidiosis from bacillary white diarrhea of young fowl (14). Beach and Corl (15) also realized in 1925 that a single exposure to coccidial infection could render chickens immune to coccidiosis. Unfortunately, these investigators made a serious error in suggesting that the severity of coccidiosis was not correlated with the number of oocysts ingested.

This was refuted in 1927 by W. T. Johnson of Oregon Agricultural College at Corvallis, who confirmed his own earlier observations that increasing numbers of oocysts did indeed produce more severe disease (85). Furthermore, in the same year, Johnson (86), and later E. M. Dickinson (31) and others, also showed that repeated inoculations with smaller numbers of sporulated oocysts could result in an acquired immunity. This foreshadowed the much later demonstration by L. P. Joyner and C. C. Norton in the 1970s of the powerful immunogenic effects of "trickle infections," i.e., very small but very frequent multiple doses of sporulated oocysts (89,90). However, despite the groundbreaking work of Beach, Johnson, Tyzzer and others, there was to be no development of a commercial anticoccidial vaccine for many years to come.

This may have been because most researchers concentrated on the chemotherapeutic approach to coccidiosis control, encouraged perhaps by some limited success in the 1920s with antihistomonal drugs (150) and amebicides (34). After some encouraging results with buttermilk (15), skim milk (16), and flowers of sulfur (69) against *Eimeria tenella*, the major breakthrough came with Prof. Pincus Philip Levine's demonstration in 1939 of the anticoccidial activity of sulfanilamide (101). According to Lund (112), Levine's paper stimulated at least 238 publications on new drugs in the succeeding 20 yr.

In 1949, a widely publicized conference on coccidiosis was organized by the New York Academy of Sciences (18). In conformity with the general approach to coccidiosis control at that time, 11 of the 26 conference papers addressed chemotherapy, whereas only one was relevant to immunity. It was in this intellectual climate that S. A. Edgar began in the 1940s to formulate an idea for a commercial anticoccidial vaccine. This was a bold decision because progress by the established experts in translating experimental results into any method of stimulating practical immunity to coccidiosis in the field had not been particularly successful. So, what was known at that time about the active immunization of chickens against coccidiosis?

EARLY PRACTICAL WORK ON COCCIDIAL IMMUNIZATION OF CHICKENS

Some work of W. T. Johnson has already been mentioned. Johnson became Professor of

Veterinary Medicine at Oregon State College and would probably have achieved even greater prominence in the field of coccidiosis had he not died in 1937 at the early age of 45. A posthumous paper (88), prepared by Johnson's widow, was prefaced by a tribute penned by Dean William A. Schoenfeld in 1938: "His work has shown that under experimental conditions chickens can be successfully immunized against five species of coccidia. Data from field trials are not available to demonstrate that these same species can be satisfactorily controlled under commercial poultry-farm conditions. It was in this particular field that Dr. Johnson's experimental studies were cut short by his untimely death, thus leaving for others the accomplishment that not only had been his chief ambition but to which he gave his entire personal resources with heroic self-effacement."

It seems that the closest Johnson came to achieving his dream was his success in 1932 in immunizing chickens by including sporulated oocysts in their feed (87). It would surely have gratified him to know that many of the influential workers who followed in his footsteps made reference to his work on immunization. They included, among others, E. E. Tyzzer, E. R. Becker, R. L. Mayhew, E. M. Dickinson, M. M. Farr, and S. A. Edgar. By the 1950s, it had become generally recognized that coccidial immunity is effectively stimulated by a series of autoreinfections initiated by a small dose of oocysts (70).

In 1932, Tyzzer demonstrated remarkable prescience during a discussion of his approach to the control of coccidiosis (152), giving credit to Johnson's previous work: "With regard to the value of drug products in the treatment of coccidiosis, we have not attached much importance to this method of attacking the problem. We have not interested ourselves in these questions since it appears to us more important to introduce infection than to keep it out. The great mistake in measures employed to eradicate coccidiosis infection appears to be that they have been too effective. Various clean-up measures are advocated—the use of the fire-gun, the employment of wire platforms, in fact, everything to combat infection—and with what result? I think that Johnson has pointed out the principle that is here concerned. The birds may develop well until they approach maturity but

sooner or later infection appears at a time when its consequences are most serious.”

The major problem to be surmounted in immunizing commercial flocks was how to develop a controlled method of oocyst administration without the risk of clinical disease occurring during the acquisition of immunity. In 1941, Dickinson used the tedious method of daily individual gavage for 5–50 days to stimulate immunity (31); clearly, this did not have any large-scale commercial potential. In the 1930s and 1940s, another general approach to achieving safe immunization was to attempt the attenuation of coccidia by heat treatment (74) or X-irradiation (7,159). Unfortunately, none of these efforts provided a solution, as observed by Jeffers (79): “In no case have any of these treatments resulted in a consistent and stable change in a strain of coccidia whereby the pathogenicity of viable parasites is reduced.” Most likely, heat or X-rays simply killed varying proportions of the oocyst population, thus limiting the dose received by the chicken. It probably seemed to Edgar, during the 1940s, that any practical process would have to depend on an efficient method of administering viable, virulent oocysts in approximately equal small numbers to each chicken, which would establish further reinfections to stimulate an immune response.

Edgar spent his early academic years at Sterling College (A.B. degree), Kansas State University (M.S. degree), and the University of Wisconsin, where in 1941 he became interested in the problem of immunization, encouraged by Chester A. Herrick (109), who was his zoology-major professor in charge of his Ph.D. studies (39,49). I was surprised to discover that, according to the records of the University of Wisconsin Library, nobody had ever read Edgar’s Ph.D. thesis of 1944 before I borrowed it in 2001. From this thesis, it is clear that Edgar was thoroughly familiar with the work of previous authors who had been able, by various means, to stimulate acquired immunity to coccidia of chickens (39). After discussing a comprehensive list of publications, he stated, “Considerable effort has been exerted by various workers to put immunization procedures on a practicable basis, and it was with this in mind that the following experiments were conducted.”

His first experiment was begun in June 1942. A key result was his confirmation that significant immunity to *E. tenella* can be developed by giving several small doses of oocysts and his conclusion that this method may allow development of immunity without mortality (39). Further work by Edgar in this field was delayed by his army service in World War II (109). After his return to the United States, some progress was made during 1947–48 at the Alabama Polytechnic Institute (API) at Auburn, but this was then interrupted by a 2-yr stint in Tahiti, working on human filariasis (109). This explains Edgar’s surprising absence from the New York Academy of Sciences coccidiosis conference in 1949 (18). He was finally able to return in 1950 to the API, later to become Auburn University, where he spent 42 yr as an educator, dedicating himself to the service of the American poultry industry, for which he received several prestigious awards.

THE SCIENTIFIC EVOLUTION OF ANTICOCIDIAL VACCINES

My recent account of anticoccidial vaccines (163) describes how their use has been adapted for broilers in recent years, using knowledge based on bird behavior, parasite biology, and epidemiology. During research for that review, it was often found that features of formulation or administration now considered to be desirable for modern anticoccidial vaccines were foreshadowed in the 1950s and 1960s during Edgar’s development of the Coccivac® vaccines. For instance, he recognized early on that, because of the virulence of the vaccinal strains, it was essential to do everything possible to achieve a uniform uptake of viable vaccine (50) and, as a safety measure, to provide chemotherapeutic protection of any birds that might remain susceptible after vaccination (49). Those two factors are to a considerable extent interdependent, and the manufacturers’ recommendations relative to their balance changed over the years, as scientific knowledge accumulated. Furthermore, it was realized that chicks should be vaccinated at the youngest age possible to establish the earliest protective immunity, particularly for broilers (163).

Ubiquity of coccidial species. The first of the Coccivac® series of vaccines (DM® Cecal Coccidiosis Vaccine, launched in 1952) was

criticized because it contained only *E. tenella* oocysts and thus would not protect chickens against other coccidian species (70). Later formulations in the series contained various numbers of species according to the class of chicken to be vaccinated (Table 1). Dorsman (38), in 1956, also criticized one of these formulations (NObiCOX®) that had recently been imported into The Netherlands because of the risk of introducing species that did not previously exist there. Furthermore, as other brands of vaccine came to be registered from the mid-1980s, some national regulatory authorities expressed similar concerns regarding other countries. These concerns stimulated research that demonstrated the presence of the generally accepted seven species of chicken coccidia in any country where they had been diligently sought (162,165). Moreover, multispecific infections seem to be the norm on a farm, rather than the exception (165).

Viability of vaccinal oocysts. Fundamental necessities for the commercial production of a live anticoccidial vaccine are the uniform viability and storage stability of the vaccinal oocysts. Unless these are achieved, all efforts to develop efficient administration methods will be wasted. Edgar understood this well, and the larger part of the claims in his 1964 patent (50) addressed these issues. He pointed out that "inocula produced by previously available procedures contained unknown or low or variable numbers of viable sporulated oocysts of *Eimeria tenella*, so that production of a critically controlled infection in chicken flocks was not obtained. Erratic and unreliable results were obtained by the use of such inocula" (50). Edgar had come to realize the importance of this as a result of his work with S. H. Waxler (109) at Wisconsin before 1942, when he discovered that Waxler's results with X-irradiation were variable because of differences in the quality of the oocyst cultures used (109).

The second key factor in commercial anticoccidial vaccine production, long-term storage of coccidial oocysts, is largely dependent on keeping them free from contamination with other micro-organisms. To achieve this, the CoccVac® vaccines have, from the beginning, been formulated in a solution of potassium dichromate, a procedure known for many years from the experiments in 1927 of W. T. Johnson on sporulation and storage of oocysts (85).

Johnson credited Dr. Philip B. Hadley of Rhode Island Experiment Station with telling him that potassium dichromate could be used for viable oocyst preservation. I was unable to trace any earlier publication by Hadley in which he mentioned this discovery. In 1925, Beach and Corl (15) were sporulating oocysts by spreading cecal contents on wet filter paper in a jar. In the same year, Beach and Davis used, instead of filter paper, an agar plate moistened with sodium chloride solution, a method suggested by H. W. Graybill (16).

Johnson's 1927 paper (85) therefore appears to be the original source publication for the use of potassium dichromate, and it is interesting to note that he correctly attributed the compound's usefulness solely to its antiputrefaction quality (83). Surprisingly, though, right up to the present time, one may often see the statement that potassium dichromate also provides the oxygen that is necessary for the sporulation and survival of oocysts. This idea was in circulation during the 1940s and Edgar repeated it in his thesis (39). In fact, it is incorrect and seems to be due to a confusion between the chemical terms "oxidation" and "oxygenation." Potassium dichromate is an oxidizing agent and reacts with reducing agents with an exchange of electrons, but in so doing it does not produce nascent oxygen and therefore does not oxygenate the oocyst medium. In these circumstances, only aeration can do this, which is achieved simply by allowing the oocysts maximum exposure to the air.

Significantly, in Johnson's original experiments (85), the best sporulation was obtained by using just enough dichromate solution to moisten thin smears of fecal material, so that the air did not have to be absorbed by a large volume of liquid. The critical factors for oocyst sporulation in a Petri dish of potassium dichromate solution were succinctly stated by N. D. Levine (98): "The potassium bichromate prevents bacterial growth which might kill the protozoa, and the thin layer is necessary so that oxygen can reach the oocysts."

Route of administration. In 1932, attempts at mass vaccination of chicks depended on mixing oocysts in a wet mash fed for up to 23 days (87). Edgar also used the feed as the vehicle for DM® Cecal Coccidiosis Vaccine in 1952. However, there were two major differences in the administration method. First, the

Table 1. Successive formulations, target birds, and administration methods of the Coccivac series of vaccines.

Trade name/formulation	Species ^A	Target bird	Route	Concomitant chemotherapy	Reference source
None	<i>Et</i>	Broilers	In feed at 3 days old	2 days sulfa drug 13 days postvacc.	54
DM® Cecal Coccidiosis Vaccine	<i>Et</i>	Broilers	In feed at 3 days old	2 days sulfa drug 13 days postvacc.	35
Coxine®	<i>Eac, Eh, En, Et</i>	Broilers	Moist feed	2 days sulfa drug 13 days postvacc.	1, 41
CocciVac®	<i>Eac, Emax, En, Et</i>	Broilers?	Moist feed at 3 days	2 days sulfa drug 13 days postvacc.	Cf. 38
NObiCOX®	<i>Eac, Emax, En, Et</i>	Broilers?	Moist feed at 3 days	2 days sulfa drug 13 days postvacc.	38
MF CocciVac® (type A)	<i>Eac, Eh, Emax, En, Et</i>	Layers	Water or moist feed	Trithiadol® day old to 5 wk	36, 145
MF CocciVac® (broiler type 3)	<i>Eac, Eh, Et</i>	Broilers	Water or moist feed	Trithiadol® day old to 5 wk	36
MF CocciVac® (broiler type 4)	<i>Eac, Emax, En, Et</i>	Broilers	Water or oral-drop	Trithiadol® day old to 5 wk	36
MF CocciVac® (type B)	<i>Eac, Eb, Eh, Emax, En, Et</i>	Breeders?	Water or oral-drop	Trithiadol® day old to 5 wk	9, 36, 145
MF CocciVac®	<i>Eac, Eh, Et</i>	“Broiler type”	Water, moist feed	Trithiadol® day old to 5 wk	2
MF CocciVac®	<i>Eac, Eb, Et</i>	Layers	Oral-drop in hatchery	Trithiadol® day old to 5 wk	3
CocciVac®	<i>Eac, Eh, Et</i>	Layers	Water	Trithiadol® day old to 5 wk	4
CocciVac® (type C)	<i>Eac, Eb, Eh, Emax, En, Ep, Et</i>	Layers	Water at 10 days	Trithiadol® day old to 7 wk	145
CocciVac® (type D)	<i>Eac, Eb, Eh, Emax, Emiv, En, Ep, Et</i>	Layers	Water at 10 days	Trithiadol® day old to 7 wk/none	5, 145
Coccivac®-T	<i>Ead, Emel, Eg, Ed</i>	Turkeys	Water	None	118
Coccivac®-B	<i>Eac, Emax, En, Et</i>	Broilers	Feed, water	2 days Amprol® 12 days postvacc.	Prod. license 1989
Coccivac®-D	<i>Eac, Eb, Eh, Emax, Emiv, En, Ep, Et</i>	Layers	Water	2 days Amprol® 12 days postvacc.	Prod. license 1989
Coccivac®-B	<i>Eac, Emax, Emiv, Et</i>	Heavy broilers	Feed, water, eye, hatchery spray	2 days Amprol® 10 days postvacc.	8
Coccivac®-D	<i>Eac, Eb, Eh, Emax, Emiv, En, Ep, Et</i>	Breeders, layers	Feed, eye spray	2 days Amprol® 10 days postvacc.	8
Coccivac®-T	<i>Ead, Emel, Eg, Ed</i>	Turkeys	Feed, water, hatchery spray	2 days Amprol® 10 days postvacc.	8

^A*Eac* = *acervulina*; *Ead* = *adenoeides*; *Eb* = *brunetti*; *Ed* = *dispersa*; *Eg* = *gallopavonis*; *Eh* = *hagani*; *Emax* = *maxima*; *Emel* = *meleagrimitis*; *Emiv* = *mivati*; *En* = *necatrix*; *Ep* = *praecox*; *Et* = *tenella*.

vaccine was administered by mixing it into the feed of 3-day-old broiler chicks after 12 hr of fasting, so the oocysts were ingested within a few hours. Second, on the 13th or 14th day after vaccination, sulfaquinoxaline was used to treat the birds for 2 days to ameliorate any post-vaccinal reactions (25,35). Such a postvaccinal therapy was apparently first tried by Seeger (135) in 1947. The development of immunity depends on the repeated completion of coccidial life cycles as chickens reinfect themselves by foraging in the litter. Essentially, the feed route has remained in use for anticoccidial vaccines ever since, though it has been adapted in various ways (163).

After the establishment of the concept of trickle infections, whereby small daily doses of sporulated oocysts are used to stimulate immunity (89,90), attempts were renewed to develop practical methods of administering low numbers of oocysts in the feed daily over a long period of time, instead of during just a few hours. In the early 1980s, it was shown experimentally that chicks could be vaccinated accurately by incorporating oocysts in a wet premix of starch paste, which was dispersed in the diet to administer a trickle dose of vaccine during about 4 wk (29). Attempts were made later in the 1980s to produce vaccines based upon oocysts dispersed in a free-flowing powder or encapsulated in tiny beads made of calcium alginate or a fat-water emulsion for mixture into chicken diets (30,84,120). Unfortunately, oocysts did not survive the high temperatures encountered in the feed pelleting and fat-spraying processes, a problem that was apparently never overcome. More recently, however, it has been found that spraying vaccine in the form of naked oocysts on the first feed that day-old chicks receive after placement is an effective single delivery technique (163).

Another administration method is the incorporation of vaccinal oocysts in a bright-green edible gel that can be placed in the chick trays at the hatchery or on feed trays in the poultry house immediately after placement (28). A further hatchery method is spraying the vaccine over trays of newly hatched chicks. The chicks probably ingest the oocysts by a combination of the direct oral and ocular routes during spraying, and indirectly by self-preening and pecking drops of diluted vaccine from their neighbors as they dry off (11). After the intro-

duction in 1998 of the hatchery spray method for Coccivac®-B, postvaccinal reactions have been considerably reduced, and the previous requirement for routine therapy with amprolium in conjunction with vaccination has now been discontinued (118). The hatchery spray method of vaccination has been largely responsible for the recent increases in broiler market penetration by anticoccidial vaccines.

Thickening agents for water administration. Just as the feed was regarded as a convenient vaccine carrier for mass oral vaccination, the drinking water was regarded as an equally useful vehicle for administration (2,3,4,5,9,36). Edgar realized that, because oocysts sink quite fast in water, a thickening agent is necessary to keep them in suspension while the birds drink from an open container such as a trough or a bell-drinker. He originally used Veegum® (magnesium aluminum silicate gel) for Coccivac® (50). For other vaccines, Keltron® (xanthan gum) has been used for Paracox® (164) and carrageenan for Immucox® (95).

Moisture content of litter. Bearing in mind the crucial role of recycling infections in the development of immunity, litter condition is important for the sporulation of oocysts. Recommendations for litter management after Coccivac® administration have changed over the years. Because moisture content was considered to be critical, water was sprayed onto the litter to maintain about 20%–30% moisture (37,72,73,145); it is now realized that this is unnecessary (8) because such levels are attained naturally, at least in temperate climates (23,164). Recent research on the effects of litter moisture on oocyst sporulation has been reviewed by Williams (163).

Early administration. Until quite recently, live vaccines were most often used for breeding and egg layer stock and in recent years were usually administered in the drinking water to chicks between about 3 and 14 days old. In order to initiate immunity as early as possible in broilers, it is necessary to vaccinate chicks with a single dose at 1-day-old (163). Such early vaccination was once thought to be impracticable, because some researchers believed that very young chicks were generally more resistant to coccidial infection than older ones, possibly because of inefficient excystation of oocysts and the immaturity of the chicks' immune system (163) or because of a passive maternal immu-

nity (48). However, as early as 1960, an applicator had been developed for individual oral-drop vaccination with CocciVac® of chicks in the hatchery (3,37). Other early work with CocciVac® also showed that chicks infected at 1-day-old are perfectly capable of mounting an effective immune response (50,149), and the principle is now generally accepted.

Despite the knowledge that CocciVac® could be used in day-old chicks, by 1964 it was recommended that layers and breeders should be vaccinated at 10-days-old (145). This was partly to fit in with various bird management procedures (145) and partly to avoid potential problems that might follow vaccination of poor quality day-old chicks (M. Eckman, pers. comm.). However, the trend is now toward vaccination of younger chicks, and various modern methods of administering vaccines to day-olds have been enumerated by Williams in a review of the technical aspects of the topic (163).

Compatibility with other vaccines. Just as there is always concern about the compatibility of a new vaccine with pre-existing others, in the 1950s questions were asked about the “vaccine reactions” that might occur with CocciVac®. In response to these concerns, Edgar recommended that multiple vaccinations should be timed so that the reactions did not occur simultaneously (42,145). It should be mentioned that Edgar was involved not only with anticoccidial vaccines but with developing vaccines for Newcastle disease, infectious bronchitis, fowl pox, Gumboro disease, and Marek’s disease (109). He was thus well aware of the possible interactions of vaccinations and was able to assure users that CocciVac® could be safely administered alongside other vaccines (42,44,145).

Concomitant chemotherapy. In the early days, the fact that much of the commercial poultry industry did not use the recently launched CocciVac® vaccines may have been due to several factors, not least of which was that commercial promotion was rather poor (J. A. Kukla, pers. comm.). Whatever the reasons, the majority of coccidiosis control programs used in the United States during the 1950s continued to be based upon anticoccidial drugs incorporated in the feed (122). However, an advance in anticoccidial vaccination was made when it was discovered that CocciVac® could immunize chickens while they were being given

various drugs prophylactically in their feed (46,47,50,148,149). Hence, the short postvaccinal therapy with sulfaquinoxaline was replaced in about 1959 by a planned immunization program comprising CocciVac® with Trithiadol® or some other subeffective drug in the feed for about 5 wk (Table 1).

In the 1960s, the older drugs with rather low potency and a cidal mode of action were superseded by extremely potent ones such as clodipol and decoquinate, with quite different biochemical modes of action and with coccidiostatic effects (64,157,158,161). Naturally, there was no possibility that CocciVac® could be used alongside chemoprophylaxis in broilers, because the vaccinal coccidia were highly sensitive to those potent new drugs. Furthermore, when used continually at recommended concentrations, drugs with true coccidiostatic activity against drug-sensitive sporozoites did not allow birds to develop immunity (163).

By the mid-1960s, therefore, the idea of the concomitant use of a vaccine and chemoprophylaxis had been generally abandoned, and the inclusion of Trithiadol® or other drugs in the feed was no longer recommended (Table 1) (128,146). A Dorn and Mitchell advertisement of 1967 urged CocciVac® users to “kick the coccidiostat habit” and to use the vaccine without any drug medication (5). However, the potent anticoccidial drugs launched during the 1960s tended to develop drug resistance fairly quickly, and by the early 1970s had become superseded by the first ionophorous drugs (114). Hence, oocyst accumulation in poultry-house litter of drug-treated chickens once more began to rise to the levels encountered several years before, during use of the older drugs. High numbers of residual oocysts might potentially cause problems if CocciVac® were being used for the first time after prophylactic drug use. Hence, the recommendation for short-term postvaccinal therapy was reintroduced in the 1980s, this time with amprolium (Table 1), until the introduction in 1998 of hatchery-spray administration made it unnecessary once again (118).

Amelioration of drug resistance by vaccines. In 1976, Jeffers (77) suggested that the introduction of massive numbers of drug-sensitive attenuated coccidia onto farms where drug-resistant field strains predominate would be a useful adjunct to planned immunization.

The hypothesis was apparently not tested with a commercial vaccine until 1989, when Mathis and McDougald (113) showed that if the drug-sensitive nonattenuated vaccine CocciVac®-T was used on turkey farms where drug resistance had been a problem, the sensitivity of the local coccidial population could be substantially restored. Subsequently, a similar effect was demonstrated with CocciVac®-B (20,21) and the attenuated vaccine, Livacox® (119). A probable explanation of the mechanism, whereby vaccinal parasites interbreed with the local wild-type population, has been proposed (163).

Such studies have given rise to the idea that drug-sensitive vaccines might play an important role in prolonging the useful life of anticoccidial drugs by using vaccination and chemoprophylaxis in rotation programs (22,61). A further possible benefit of such an approach using attenuated vaccines might be that not only would drug-resistance be ameliorated but so would the virulence of the resident parasite population (160).

THE WORLD'S FIRST ANTICOCIDIAL VACCINE

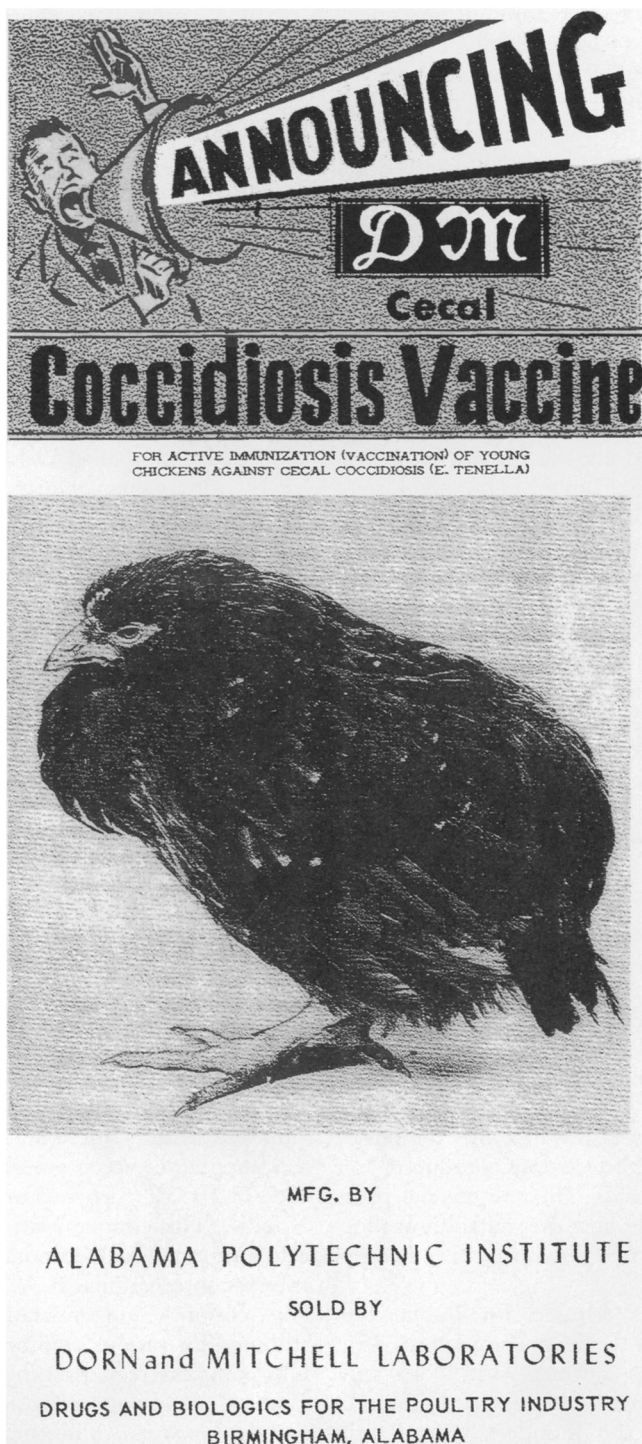
Various attempts at experimental immunization against coccidiosis during the 1930s to 1950s, which were all successful to some degree, included those by Johnson (87), Seeger (135), Goldsby and Eveleth (65), and Dickinson and his colleagues (32,33). Goldsby and Eveleth (65) even went as far as describing a method for the preparation of a virologically and bacteriologically sterile vaccine. Although all the above-mentioned researchers were able to demonstrate the scientific principle of anticoccidial vaccination, none of them ever developed a commercially practicable program. In fact, the distance between demonstrating the scientific principle and finding a way to make it useful to the commercial poultry producer proved to be vast.

Work on the development of a commercial anticoccidial vaccine had begun at the API at Auburn in 1947 (42,45), and by 1951 (24 yr after Johnson's original report on immunity [86]), success seemed to be in sight (60). Mid-1952 saw publication of a press release from the API announcing a vaccine that contained live, sporulated oocysts of *E. tenella* (70). Although there are earlier references than this to experi-

mental immunization, there is no doubt, therefore, that the first commercial anticoccidial vaccine was that launched by Edgar in 1952 (57,70). Originally, it was known as "DM® Cecal Coccidiosis Vaccine." The front page of an early promotional leaflet (35) is shown in Fig. 2. Subsequent modifications of this vaccine were marketed under the names of Coxine®, NObiCOX®, or CocciVac®.

Initial responses from poultry specialists, particularly from Oregon and California, were not especially favorable. Dr. Arnold S. Rosenwald (Agricultural Extension Service, University of California), in a press announcement on July 3, 1952, stated, "It occurs to me that immunization in cecal coccidiosis is somewhat of a waste of time since it is the one species which is the most readily and effectively controlled by either of the sulfonamides, some of the other drugs, or by special sanitation" (70). W. R. Hinshaw (70) agreed with this and also pointed out that *E. tenella* is only one of the species that affects chickens, that Edgar's technique was essentially a modification of that of W. T. Johnson (86), and that careful control would be necessary to avoid acute coccidiosis outbreaks due to overdosage.

To be fair to Edgar, the criticisms that he attracted 50 yr ago should be viewed with the advantage of hindsight. Thus, taking them in order, it was not to be long before Walertzky and his colleagues (155) discovered, in 1954, sulfonamide resistance in a field strain of *E. tenella*, a phenomenon almost immediately confirmed experimentally by Cuckler and Malanga (27); hence, the need for an *E. tenella* vaccine to control field strains refractory to drugs was soon justified. Regarding the number of species in the vaccine, Edgar had, in fact, already written (August 18, 1952) to Hinshaw (70), pointing out that preliminary results were available to show that two or more species of coccidia could produce immunity against those included in a vaccine, and, indeed successive vaccines developed at the API were to confirm this. Addressing the implied charge of lack of originality, Edgar acknowledged several times the previous work of W. T. Johnson and others (39,41,42,48,49,51). Most importantly, he demonstrated an understanding of why Johnson's original studies did not result in a commercial vaccine by pointing out that sulfonamides were not available in the 1920s to con-




ANNOUNCING

DM

Cecal

Coccidiosis Vaccine

FOR ACTIVE IMMUNIZATION (VACCINATION) OF YOUNG CHICKENS AGAINST CECAL COCCIDIOSIS (E. TENELLA)



MFG. BY

ALABAMA POLYTECHNIC INSTITUTE

SOLD BY

DORN and MITCHELL LABORATORIES

DRUGS AND BIOLOGICS FOR THE POULTRY INDUSTRY
BIRMINGHAM, ALABAMA

Fig. 2. The front page of an early advertising leaflet for DM® Cecal Coccidiosis Vaccine (35), probably 1952 or 1953.

tol postvaccinal reactions before full immunity could be established (40,49). Finally, Edgar fully understood the importance of vaccinal oocyst viability, the epidemiology of coccidiosis, and chick behavior in achieving uniform dosing, as demonstrated by his continual modifications of the vaccines, their methods of administration, and concomitant drug use.

THE COMMERCIAL EVOLUTION OF THE COCCIVAC® VACCINES (1952–2002)

DM® Cecal Coccidiosis Vaccine. The first batches of Edgar's original *E. tenella* vaccine, used for the early development work and field trials, were prepared in the basement of his home in Auburn, AL (Table 2) (J. A. Kukla, J. J. Giambrone, and H. N. Lasher, pers. comms.). At that time (1951–52), the vaccine was naturally in very short supply (35,70), and the plan was that production would be scaled up at the API and distributed by Dorn and Mitchell, Inc., in Birmingham, AL, and Gainesville, GA (25,70). Dorn and Mitchell was incorporated specifically to market the vaccine (71), and DM® Cecal Coccidiosis Vaccine was their trade name.

The new facility at the API was apparently not in full production until probably late 1954 (6,42), and until then a stopgap production unit had to be found because demand for the vaccine had outgrown the limitations of Edgar's basement. The ideal building needed to have a large number of small rooms for the efficient isolation of donor chickens and oocyst preparation. Edgar found that an empty building in Birmingham, previously used as a "house of ill-repute," served the purpose admirably (H. N. Lasher, pers. comm.)! It was here that the DM® Cecal Coccidiosis Vaccine was produced for about a year and a half. The commercial pack included the vaccine and the sulfaquinoxaline required for the standard postvaccinal therapy (35).

The vaccine was released for public use through the Auburn Research Foundation (45), which established a contract with Dorn and Mitchell, who also used other agents (Table 2). Ira Dorn, one of the founders with Ralph Mitchell, wanted to sell the new vaccine directly to consumers, but Mitchell and Edgar preferred to use a network of agricultural distributors (J. A. Kukla, pers. comm.). Interstate

sale of the product was authorized by the Pure Food and Drug Administration, probably early in 1954 (6,41). From 1952 to 1956 inclusive, the average annual sales in the United States and abroad comprised about 10 million doses (41,42,44,45,57). The price of the original formula was about 1 cent per dose (25,45).

Coxine®. By the time the new API facility (Fig. 3) came into production, Edgar had added more *Eimeria* species to the original vaccine. He had been trialling a multivalent vaccine since about mid-1953, and it was released late in 1954 (6) as Coxine®, which soon superseded the original DM® Cecal Coccidiosis Vaccine. Coxine® was manufactured at the API and distributed by the Gland-O-Lac Company, Omaha, NE, an agent of Dorn and Mitchell (Table 2). It contained *E. acervulina*, *E. hagani*, *E. necatrix*, and *E. tenella* (1), and was therefore the first multivalent vaccine for coccidiosis. Whether Coxine® was a trade name of Dorn and Mitchell or of Gland-O-Lac is not certain.

The API production facility was a state-of-the-art laboratory designed jointly by Prof. Dale Franklin King and Edgar (42). Vaccine was produced in conformity with the regulations of the Bureau of Animal Industry (BAI), although sale of the vaccine did not come under the authority of that agency (42). The BAI was a division of the United States Department of Agriculture, now superseded by the Animal and Plant Health Inspection Service (APHIS). By 1956, an estimated 15 million doses of Coxine® had been sold in the United States and abroad (44). The price ranged between 1 and 1.5 cents per bird, depending on the quantity purchased (44).

NobiCOX®. Soon after Coxine® was launched in the United States during 1954, the first supplies of vaccine were imported into Europe in 1955 (38) by a Dutch company called Nobilis. (This company, after a merger with another international corporation, was renamed Intervet International B. V. [T. M. P. Schettters, pers. comm.], and the trade name Nobilis® is still used for various poultry vaccines.) The Nobilis company requested the API to modify the Coxine® formulation by replacing *E. hagani* with *E. maxima*. This new formulation was marketed in Europe by Nobilis under the name Coccivac®, except in Belgium and The Netherlands, where the name NobiCOX® was introduced, because Coccivac® was similar to an-

Table 2. Manufacturing sites and distributors of the Coccivac series of vaccines (all in the United States unless otherwise stated).

Trade name/formulation	Manufacturing site	Distributor	Dates
None	S. A. Edgar, Auburn, AL	Auburn Research Foundation	1951–52
DM® Cecal Coccidiosis Vaccine	“House of ill-repute,” Birmingham, AL	Dorn and Mitchell, Inc.	1953–54
Coxine®	API, Auburn, AL	Gland-O-Lac Company	1954–59
CocciVac®	API, Auburn, AL	Nobilis (The Netherlands)	1955–?
NObiCOX	API, Auburn, AL	Nobilis (The Netherlands)	1955–?
MF CocciVac® (type A)	Opelika, AL	Dorn and Mitchell, Inc., DPL ^A	1959–?
MF CocciVac® (broiler type 3)	Opelika, AL	Dorn and Mitchell, Inc.	1959–66
MF CocciVac® (broiler type 4)	Opelika, AL	Dorn and Mitchell, Inc.	1960–84
MF CocciVac® (type B)	Opelika, AL	Dorn and Mitchell, Inc., DPL	1960–84
MF CocciVac® (type C)	Opelika, AL	Dorn and Mitchell, Inc., DPL	1964–?
CocciVac® (type D)	Opelika, AL	Dorn and Mitchell, Inc., DPL	1964–84
CocciVac®-T	Opelika, AL	Dorn and Mitchell, Inc.	1984
Coccivac®-T	Millsboro, DE	Sterwin Laboratories	1985–97
Coccivac®-B	Millsboro, DE	Sterwin Laboratories	1989–90
Coccivac®-B (relicensed)	Millsboro, DE	Sterwin Laboratories	1991–97
Coccivac®-D	Millsboro, DE	Sterwin Laboratories	1985–97
Coccivac®-B	Millsboro, DE	American Scientific Labs	1998–99
Coccivac®-D	Millsboro, DE	American Scientific Labs	1998–99
Coccivac®-T	Millsboro, DE	American Scientific Labs	1998–99
Coccivac®-B	Millsboro, DE	Schering-Plough Animal Health	2000-present
Coccivac®-D	Millsboro, DE	Schering-Plough Animal Health	2000-present
Coccivac®-T	Millsboro, DE	Schering-Plough Animal Health	2000-present

^ADPL = Delaware Poultry Laboratories.



Fig. 3. The coccidiosis laboratory and vaccine production unit at Alabama Polytechnic Institute. The building still exists, and this photograph by S. H. Fitz-Coy was taken in 2001.

other protected trade name (T. P. M. Schettters, pers. comm.). In 1956, Dorsman concluded that there was little need for a vaccine in The Netherlands (38), and this may have influenced potential users. Because of a lack of wide acceptance in Europe, the product was withdrawn from the market, but the date is uncertain.

CocciVac®. The first use of the name CocciVac®, a trade name of Dorn and Mitchell, seems to have been in Europe in 1955 (archives of Intervet International B. V.). In the United States, the first mention found was in Dorn and Mitchell's 1959 brochure entitled *Technical information and review of experimental data—CocciVac* (36), but it may well have been used earlier. By about 1960, production and packaging of CocciVac®, under the direction of Edgar with Joe A. Kukla as production manager, had been transferred to a much larger factory in Opelika, AL (Table 2), on the corner of Highways 280 and 147. The building is currently an antiques store (J. A. Kukla, pers. comm.).

In 1958, Sterling Drug, Inc., acquired Dorn and Mitchell (Fig. 4) in order to complement their anticoccidial drug Trithiadol®, which was used subsequently in combination with CocciVac® (149). Dr. Frederick Coulston, of the Sterling Winthrop Research Institute in

New York, soon recommended that Sterling Drug also purchase Delaware Poultry Laboratories (DPL) at Millsboro, DE (Fig. 4) to strengthen the product range and marketing and technical support capabilities of the company (H. N. Lasher, pers. comm.). DPL then shared marketing of CocciVac® with Dorn and Mitchell (145). This brought the vice-president of DPL, Dr. Hiram N. Lasher, into the picture, an appropriate appointment because he had studied under Prof. P. P. Levine at Cornell University during 1938–42 when Levine was largely occupied with coccidiosis research (109). In 1970, when Lasher became president of Sterwin Laboratories, Inc., he became involved with the CocciVac® production at Opelika, concentrating particularly on biosecurity, oocyst harvesting, formulation, and purity issues (H. N. Lasher, pers. comm.).

After the acquisition of Sterwin Laboratories by the International Mineral Corporation, Inc., in 1984, the production operation of CocciVac® was transferred in 1985 to Millsboro, DE (71), when Kukla was succeeded as production manager by A. A. (Fred) Alls. Millsboro was the future site of the Sterwin Division of Pitman-Moore (Table 2, Fig. 4), under the vice-presidency of Dr. Fred W. Melchior, Lasher having resigned in 1979 (71). The site then served suc-

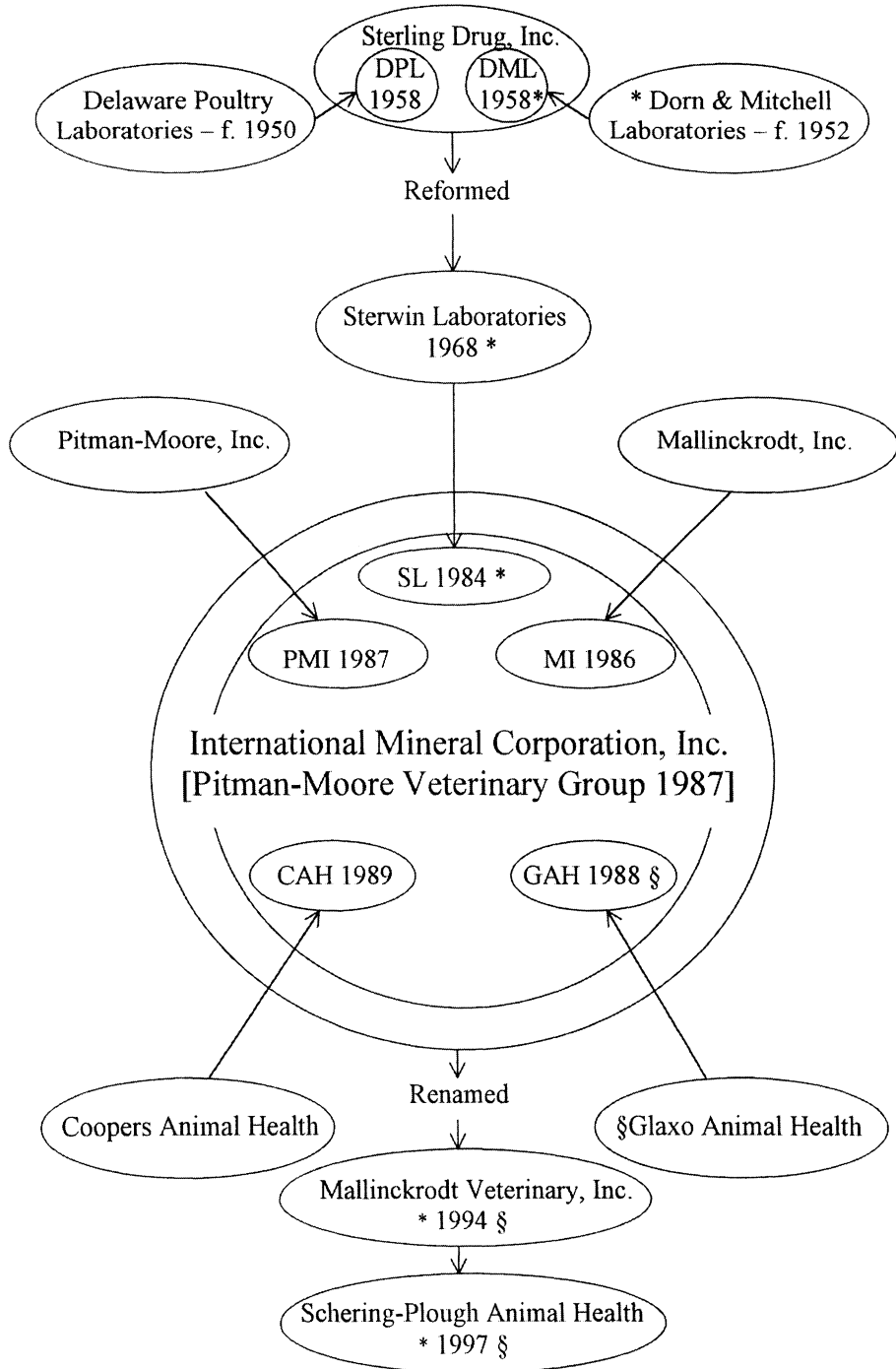


Fig. 4. Organogram tracing the ownership of marketing rights for the CocciVac (*) and Paracox (\$) vaccines through the historical development of various animal health companies (25,71, and pers. records).

cessively as the base for Pitman-Moore, Malinckrodt Veterinary, American Scientific Laboratories (a division of Schering-Plough), and Schering-Plough Animal Health (Table 2, Fig. 4). The administrative buildings were the very same as those built for Lasher's DPL and are still in use today under the Schering-Plough banner.

Fig. 4 shows how the marketing rights of the Coccivac® series of vaccines were passed through the ownerships of successive companies. Unfortunately, records of sales after 1956 are lost, doubtless discarded during the many company changes. Of the clues that exist, Edgar stated in a letter that 25 million doses had been ordered during February 1959, alone. Although Coccivac® may have been used in other countries, anticoccidial vaccination programs were not widely accepted (38). Coccivac® was imported into Europe (where in some countries it was also known as NOBiCOX) from 1955 (38), although not for very long. In 1982, the adoption of the vaccination program was still rather limited outside the United States (62), but Coccivac® had been used in Rhodesia (now Zimbabwe) during the 1960s and 1970s (72,73). An idea of the rapidly developing market may be obtained, however, from the fact that over 1.5 billion doses of Coccivac® vaccines were sold worldwide during 2001 (L. Manogue, pers. comm.). The current formulations of Coccivac® as licensed in 1989 and 1990 are Coccivac®-B, Coccivac®-D, and Coccivac®-T. They are now registered in 26, 25, and 3 countries, respectively.

Coccivac® for turkeys. The date of introduction of Coccivac®-T was at first rather difficult to determine because I did not find any advertisements for it, and it is not listed in any of the Dorn and Mitchell brochures reviewed to date. From Edgar's personal notes and letters, it is certain that he was carrying out field trials as early as 1958, and he referred to Turkey Coccivac® in several of his letters to collaborators. However, sometimes the composition mentioned was *E. adenoides* and *E. meleagritidis* and sometimes those two species with *E. gallopavonis*. It is not clear from those letters whether Edgar was writing about different versions of commercially available products or experimental formulations. Edgar and Bond published a paper in 1960, entitled "Now—a vaccine for coccidiosis in turkeys" (54), in which

a projected launch date of January 1961 was given, but no corroborative evidence has been found for the existence of a product at that time. It seems that none of these references to turkey vaccination resulted in a commercial vaccine because Fayer and Reid in 1982 (62) stated that such a product was still a desideratum, and it was not until 1989 that Mathis and McDougald (113) described Coccivac®-T as "recently introduced in the USA."

The first unequivocal evidence for the availability of a commercial vaccine is provided in a conference paper presented in 1985 by Edgar, who explicitly mentioned commercial immunization of turkeys (52). Poss (121) gave a date of 1984 for the launch of Coccivac®-T in the United States, the year before the move of the production facilities from Opelika to Millsboro (Table 2). This launch date is confirmed in a technical manual issued in 1998 by American Scientific Laboratories (8) (Table 2). The species included in the vaccine formulation are given as *E. adenoides*, *E. dispersa*, *E. gallopavonis*, and *E. meleagritidis* in a new product license issued to Sterwin in 1989 (G. P. Knight, pers. comm.), and they remain unchanged (8).

Nomenclature of Coccivac® formulations. Over the years, many versions of the Coccivac® vaccines were marketed both for chickens and for turkeys (Tables 1, 2). At least seven formulations were available for chickens between the mid-1950s and 1967. The nomenclature of these formulations is rather confusing. Originally, there was a capital "V" in the middle of the trade name (Coccivac®), but this was changed to lower case (Coccivac®) about 1985 when production was moved to Millsboro. The name Coccivac® was often preceded by the initials MF, at least up to the 1960s; this probably referred to the then current route of administration, "moist feed," although the vaccine could also be administered in the drinking water (Table 1). The curious mixture of upper and lower case letters in the trade name NOBiCOX® (which belonged to the Dutch company, Nobilis) was confirmed from the archives of Intervet International B. V. (T. P. M. Schettters, pers. comm.).

The capital letters used to designate the types of chicken vaccines, e.g., Coccivac®-B, are not abbreviations but refer simply to Edgar's laboratory codes for the species combinations (S. H. Fitz-Coy, pers. comm.). The "T" in Coccivac®-

T, however, stands for “turkey.” The early chicken types identified by numerals seem to correlate with the numbers of species in them, e.g., broiler types 3 and 4 (Table 1). A further complication is that over time, the compositions of some types were changed, or the name of a particular composition was changed. For instance, the species combinations in the CocciVac®-B formulations of 1960, 1989, and 1998 are all different from each other, but the composition of the 1989 CocciVac®-B is, in fact, the same as that of the 1955 CocciVac®/NOBiCOX® and the 1960 CocciVac® broiler type 4 (Table 1). However, the CocciVac®-D formulations of 1964, 1989, and 1998 all contain the same species (Table 1).

Curiously, Edgar very rarely used the trade name CocciVac® in his scientific or even his popular publications (I found only two examples). Perhaps this was an attempt to maintain the reputation of an independent scientist by distancing himself from commercial promotion in his research papers.

The CocciVac® patent. Considering the many formulations of the CocciVac® vaccines from 1952 onward, it is surprising that only one relevant patent could be traced, and that was granted as late as 1964 (50). Examination of this patent revealed that it covers only the derivation of viable *E. tenella* oocysts and their use as vaccinal material. No mention is made of a commercially available vaccine. Why did it take so long to obtain a patent on what was effectively the original DM® Cecal Coccidiosis Vaccine, and why were no patents obtained on any of the subsequent multivalent formulations?

The Patent File History shows that the application was filed on April 3, 1961. The apparent lateness of this application is explained by the patent attorney’s covering letter, which states that “This application is a continuation-in-part of the inventor’s copending application Serial No. 814,658, filed May 21, 1959, which is a continuation-in-part of the inventor’s prior application Serial No. 358,959, filed June 1, 1953 and now abandoned.” It is further recorded that the examiner rejected the 1961 application on August 15, 1961, on the basis of prior art revealed by the publications of E. M. Dickinson and his colleagues in Corvallis, OR. This must have caused considerable anguish to Edgar. He did not, however, give up, and he

submitted a challenge to the examiner’s decision on February 13, 1962. This elicited a final rejection on March 27, 1962. But Edgar appealed on September 25, 1962, and finally, on January 15, 1964, he received his patent.

The drawn-out battle over his patent application must have rather exhausted and frustrated Edgar because he wryly noted in his entry in *Profiles of Coccidiologists* (109), “Patents issued: One (applied for 8, allowed only 1 to be issued).” In practice, little would have been gained by applying for further patents on later CocciVac® formulations because of Edgar’s own prior art. The real commercial protection of such vaccines lies in the production know-how. In this respect, Edgar was an important influence on CocciVac® almost to the end of his life, still being a consultant to Mallinckrodt Veterinary, Inc. until 1994 (F. W. Melchior, pers. comm.). However, why the granting of this patent took 11 yr remains a mystery.

RECENT COMMERCIALLY AVAILABLE ANTICOCIDIAL VACCINES (1985–2000)

The following section deals with those well-documented vaccines other than CocciVac® that are or have been available commercially from 1985 to 2000. There is apparently some small-scale use of locally produced anticoccidial vaccines in various parts of Asia (144), but reliable information has been hard to find. An up-to-date summary of some experimental vaccines subsequently in development or near to market is provided by Williams (163).

Nonattenuated, drug-sensitive vaccines.

It was not until 1985 that a second brand of commercial vaccine was launched to compete with the CocciVac® series. This was Immucox®, developed by Dr. Eng-Hong Lee (109) and marketed first in Canada, then in other countries, by Vetech Laboratories, Ltd. (96). Formulations are available both for chickens and turkeys and have been registered now in 40 countries. These vaccines are basically similar in concept to the CocciVac® series. However, the administration methods are rather different, in particular the incorporation of vaccine in gel “pucks” that may be consumed by chicks in transit to farms (163).

Nonattenuated, drug-resistant vaccines.

In 1989, a vaccine containing only oocysts of

E. maxima was manufactured specifically for the control of *E. maxima* field strains that were partially or fully resistant to ionophorous anticoccidial drugs (134,140,142). This vaccine, called VAC M[®], was based upon the patents of Drs. K. W. Bafundo and T. K. Jeffers (10,11) of the Lilly Laboratories. It was manufactured for only a short time, probably less than 2 yr, by Sterwin Laboratories, Inc., for distribution on a limited basis by Elanco Products, Inc. (A. A. Alls, pers. comm.). Detailed discussion of the mode of action of this vaccine, which was used in the presence of ionophore-medicated feed to provide protection against subsequent natural exposure to other species, is reviewed elsewhere (163).

Some confusion exists in the literature about whether the VAC M[®] *E. maxima* was ionophore sensitive (140,142) or resistant (134). Although the patents of Bafundo and Jeffers cited examples with an ionophore sensitive strain of *E. maxima*, Williams (163) was able to confirm, after consultation with Jeffers, that the VAC M[®] strain was actually ionophore resistant. The development of a similar combination of a non-attenuated, ionophore-resistant vaccine (Nobilis[®] COX ATM) with ionophore-medicated feed was announced in 1999 by Intervet International B. V. in Europe (132).

Attenuated by selection for precocity, drug-sensitive vaccines. Because Jeffers was never in a position to capitalize on his discovery of precocious parasites (75), the field was left open for anybody to use such lines in a vaccine. Hence, in 1981, Drs. Martin W. Shirley (109) and Vincent McDonald and colleagues at the HPRS (a research station of the former Agricultural and Food Research Council) in the United Kingdom embarked on a program of work that ultimately led to a multivalent vaccine based on precocious lines of seven *Eimeria* species of the chicken (139). In 1982, the HPRS began a joint project with Glaxo Animal Health Ltd. in the United Kingdom, mediated by the British Technology Group, to develop the vaccine.

Responsibility at Glaxo for field trials and working up methods for vaccine production was then taken on by Dr. Brian Roberts and Graham P. Knight. During that period, the field work was carried out in collaboration with Shirley and McDonald of the HPRS. Chris C. Norton (109) and Janet Catchpole (109) of the

Central Veterinary Laboratory (then part of the Ministry of Agriculture, Fisheries and Food) were later involved in floor pen and shelf life studies. After the acquisition by Pitman-Moore of Glaxo Animal Health Ltd. in 1988 and Coopers Animal Health Ltd. in 1989, Dr. Ray B. Williams (109) assumed responsibility for the scientific aspects of process improvement, registration of the vaccine under license, and further development of the vaccine.

The vaccine became known as Paracox[®] and was first marketed in 1989 in The Netherlands. After a series of further reorganizations and company acquisitions, Paracox[®] is now marketed by Schering-Plough Animal Health, based in Union, NJ. Fig. 4 traces the ownership of marketing rights through successive company changes. The definitive description of Paracox[®], its development and biological characteristics, was published by Williams (160), and another formulation, Paracox[®]-5, developed specifically for broilers (63), became available in 2000. To date, about 660 million doses of Paracox[®] and 370 million doses of Paracox[®]-5 have been sold. Paracox[®] is registered in 33 countries and Paracox[®]-5 in 19 countries.

Attenuated by embryo adaptation, drug-sensitive vaccines. Apart from precocious lines, the only other type of attenuated coccidium used in a commercial vaccine is an embryo-adapted line of *E. tenella*; this is combined with precocious lines of other species in the Livacox[®] vaccines (17,141,163). These vaccines were developed by Dr. Petr Bedrník and launched in the Czech Republic by Biopharm in 1992. Technical details of the Livacox[®] vaccines are provided by Bedrník (17).

By 1997, Livacox[®]-T had achieved sales of 60 million doses (141). Since then, a further 360 million doses of Livacox[®]-T have been sold, and since 1999, 80 million doses of Livacox[®]-Q. These vaccines are registered in 24 countries. Livacox[®]-D was only ever available in the Czech Republic and has now been withdrawn (P. Bedrník, pers. comm.).

ANTICOCIDIAL VACCINE SCIENCE: PEOPLE AND EVENTS (1952–2002)

S. A. Edgar's influence on anticoccidial vaccine research. These days, it seems to be almost universal that the introduction of a new product meets with some commercial resis-

tance. Edgar's new vaccine was no exception. As already pointed out, the announcement of the first *E. tenella* vaccine was received with antagonism, especially on the West Coast of the United States, maybe because of a feeling of loyalty to W. T. Johnson, who perhaps would have got there first if not for his early demise.

Nevertheless, Edgar had made his case on sound scientific grounds while giving due credit to Johnson and others, and although he had now taken a clear lead, researchers in Oregon and California nevertheless doggedly continued the chase. One cannot help but suspect some degree of bias in the resulting papers (12,32), which pointedly acknowledged the early work of W. T. Johnson, E. E. Tyzzer, and E. M. Dickinson while ignoring Edgar's work and omitting any reference to the CocciVac® vaccines that had already been on the market for 7 yr. Furthermore, these publications added little to what was already known at that time about coccidial immunization, and no commercial vaccine resulted from the studies.

Edgar, on the other hand, had by then firmly established his expertise in the field. Building on his Ph.D. results, he continued expanding his knowledge of parasite biology, prevalence, host-parasite relationships, development and duration of immunity, and epidemiology. From this he was able to state that early immunization was advantageous because 1) young chicks are the least valuable, 2) young chicks are least susceptible to coccidiosis, 3) there is the least effect on weight gain and feed conversion efficiency, 4) this period is before natural outbreaks occur, 5) this period is before the usual stresses that occur during the most rapid growing period, and 6) if anticoccidial therapy is required, medication costs less for young chicks than for older birds (37,43,44).

Edgar constantly strived to improve his understanding of how live vaccines worked. As a result, the many changing formulations of the CocciVac® vaccines and recommendations for their use over the last 50 yr may seem confusing (Table 1), but they were the result of constantly developing scientific knowledge. Early examples of changes in concomitant chemotherapy have already been given; later, when ionophore use was well established, Edgar once again examined vaccination with prophylactic chemotherapy, this time using monensin (55). However, this did not result in the reintroduction of con-

comitant chemotherapy with CocciVac®, and currently none is recommended.

Edgar's relationships with other researchers. On the basis of the consensus of several people interviewed for this history, confirmed by my own experiences, Edgar might fairly be described as having a very strong, even overbearing, personality. This characteristic, combined with a mental toughness and persistence together with a secretiveness where proprietary matters were concerned, apparently did not endear him to many. His habit during scientific meetings of dismissing a newly presented piece of work as something that he had already done some years before but had not published was certainly frustrating (L. R. McDougald, pers. comm.). Indeed, much of Edgar's work was never published because of his desire to protect his interests in CocciVac® (S. H. Fitz-Coy, pers. comm.), and the resulting paucity of papers on the vaccine's field performance has already been noted (163). Despite undoubted difficulties with relationships in the scientific community, Edgar exerted considerable influence in the poultry industry and was certainly expert at marketing his inventions.

A keen professional rivalry existed between Edgar and Prof. W. Malcolm Reid (109) of the University of Georgia at Athens. Reid, whose reputation rested mainly upon his work on anticoccidial chemotherapy, was considerably frustrated by being unable to find out the precise formulations of the CocciVac® vaccines, but Edgar would never reveal them (R. N. Brewer, pers. comm. Prof. Brewer was well placed for this observation, for he gained his M.S. with Edgar and his Ph.D. with Reid). Despite this, Edgar seemed happy to supply Reid and others with oocyst samples for experimental infections, as indicated by the acknowledgments to this effect in many papers that emanated from the Georgia Poultry Department and elsewhere.

Having spent a week with Edgar at Auburn in early 1973, I found him to be an enthusiastic teacher, eager to impart knowledge to a young man with a brand new Ph.D., though rather dogmatic and not too disposed to discuss the possibility of any different interpretations of his opinions. This personal impression is in accord with that of others who worked with him in the early days of CocciVac® production (J. A. Kukla, pers. comm.). Nevertheless, his teaching

methods produced 51 successful M.S. and Ph.D. students (109). Whereas Edgar's reputation in the coccidiosis field was generally well respected, there were some who believed that his opinions on virology were not quite so reliable (J. J. Giambrone, pers. comm.).

Edgar and the *Eimeria mivati* controversy. Perhaps the most controversial aspect of Edgar's research was his description, with C. T. Seibold in 1964, of *E. mivati* in the chicken (59). Edgar had spent some years working on this nominal new species and had presented a paper on it at the August 1961 meeting of the American Society of Parasitologists (ASP) (58), although he did not then name the species. However, from a letter dated June 29, 1961, to Ira Dorn, President of Dorn and Mitchell, it is clear that Edgar had, in fact, already decided on the name *E. mivati* for this new species, and he was eager to include it in at least one formulation of CocciVac®. Oocysts designated as *E. mivati* were included in CocciVac®-D in 1964 (145) as soon as the new species description was published (Table 1).

Reid must have obtained a sample of *E. mivati* from Edgar during late 1960 or early 1961 in order to conclude from challenge experiments that the new unnamed species was apparently the most common in American broilers (127); this paper immediately followed Edgar and Seibold's (58) in the 1961 ASP meeting. In 1963, Reid spent several months in Europe trying to find *E. mivati* in commercial chickens. Copies of letters in my possession show that Edgar supplied samples to Reid for this trip, and he authorized Reid to pass them on to Michael L. Clarke of the Wellcome Foundation in the United Kingdom to help with the work there.








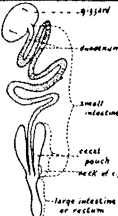
Thus, even before a scientific description was published, there was considerable effort to demonstrate that the new species existed outside the United States, but doubts later arose for some of those foreign workers who were at first convinced of the validity of *E. mivati*. The ensuing controversy, still continuing, may be encapsulated in a few of the papers that were generated regarding the conflicting evidence surrounding the taxonomy of *E. acervulina*, *E. hagani*, *E. mitis*, and *E. mivati* (13,56,91,105,130,136,137,138,143).

Edgar, Reid, and the "diagnostic chart." Most coccidiologists are familiar with the well-

known diagnostic guide to fowl coccidial species, several editions of which were authored by W. M. Reid and issued by the University of Georgia College of Agriculture (110,123,124,126). The chart of the characteristics of fowl coccidia, with its stylized chicken intestines showing the sites of development of each species, has been adapted many times for use in popular articles and brochures issued by animal health companies (125). The general assumption, certainly by the researchers that I have questioned, seems to be that the original design of the chart was Reid's. The first edition of the chart (123) acknowledged five named authors "and others" as sources of information, but through the editions and reprints up to 1984, these acknowledgments became reduced to "compiled from various sources."

I have discovered that, in fact, the chart including the stylized drawings was based upon several versions that were developed by Edgar, beginning as early as 1959 in brochures (36,37) that he designed for Dorn and Mitchell (see Fig. 5 for a 1960 version) and appearing in modified form in his description of *E. mivati* (58), the same year (1964) in which Reid first published his diagnostic guide (123). Edgar sent Reid a typescript of his *E. mivati* paper on May 6, 1963, about a year before it was published (relevant letters of Edgar and Reid are in my possession), and Reid must also have seen the Dorn and Mitchell brochures. In 1964 (145), Edgar improved his chart design by adding diagrams of oocysts, and it seems no coincidence that Reid followed suit in the next edition of his version of the chart (124).

The Reid diagnostic guide thus provides a classic example of that phenomenon of scientific publishing whereby the origins of ideas and terminology become progressively blurred until they are traceable only by backtracking through the several editions of a work and their contemporary literature. Besides the true origin of the diagnostic chart design, credit for the term "coccidiasis" is also obfuscated in Reid's diagnostic guides. The first (1964) edition (123) contains a section on "Coccidial infection *vs.* coccidiosis" in which the distinction between clinical and subclinical infections is discussed. In 1968, the word "coccidiasis" was substituted for "coccidial infection" (124), with no definition or clue as to its then fairly recent source, although later editions (110,126) subsequently

Characteristics for Differentiating the Species of Chicken Coccidia								
CHARACTERS	<i>E. tenella</i>	<i>E. necatrix</i>	<i>E. brunetti</i>	<i>E. maxima</i>	<i>E. acervulina</i>	<i>E. hagani</i>	<i>E. praecox</i>	<i>E. mitis</i>
Shape	Egg-shaped	Oblong-ovaloid	Egg-shaped	Egg-shaped	Egg-shaped	Broodily ovoid	Ovoidal	Subspherical
Size in microns	Av. 22x19	Av. 20.4x17.2	Av. 24.6x18.8	Av. 30.5x20.7	Av. 18.3x14.6	Av. 18.1x16.5	Av. 21.3x17.1	Av. 16.2x16.0
Principle Location in Digestive Tract								
Macroscopic lesions and exudate	Hemorrhagic ceca, thickened wall, bloody	Hemorrhagic & whitish exudates, thickened wall, bloody mucoid	Petechiae, catarrhal enteritis, with blood-tinged exudate, coagulative necrosis	Petechiae or thickened wall, pinkish mucoid	Petechiae, white transverse bands, wall slightly thick	Pin-head severe hemorrhages, severe catarrhal inflammation	None, except mucous coats, watery	Petechiae and white round lesions
Pathogenicity* Mortality~ Morbidity~	XXXX XXXX	XXXX XXXX	XX XXXX	XX XXXX	X XXX	X XX	X	XX
Occurrence	Very Common	Common	Occasional	Very Common	Very Common	Common	Common	Common
Duration of infection	10 days	8-12 days	8-10 days	9 days	6 days	8 days	6 days	7 days

*X = degree of pathogenicity. XXXX = highest degree of pathogenicity.

Fig. 5. The 1960 version of Edgar's diagnostic chart that appeared in Dorn and Mitchell's *CocciVac® Service and Information Manual* (37).

expanded on its interpretation. Who would have guessed from this that Prof. Norman D. Levine (98) coined the word in 1961?

It is not my intention to suggest that Reid purposely appropriated the ideas of other workers; it simply seems that he sometimes used convenient information or new terms without giving much thought to their sources, when it might have been suitable to do so. Even when in 1990 he gave credit (125) to Levine for coining the term "coccidiosis", he did not correctly identify the original reference! Ever since I first met Reid in 1973, I noticed similar mild lapses; I have letters between him and me discussing a similar issue of overlooked original sources in one of his papers. When such things were pointed out, he was always most apologetic, and I found him to be an honest and kindly man who would normally be quick to give credit where due. I address these issues here only to prevent any future misunderstandings by researchers new to the field, because these issues are important aspects of the literature on

coccidiosis in general and the history of vaccine development in particular.

T.K. Jeffers and precocious *Eimeria* strains. After the development of the CocciVac® and Immucox® vaccines, it was natural that attempts should be made to develop an attenuated vaccine. Early work on heat treatment or X-irradiation of oocysts had been unsuccessful, but in 1974, one of the most important papers in coccidiosis research in the 20th century was published. This was Dr. T. K. Jeffers's seminal study of the selection of precocious mutants from coccidial populations (75). After further elucidation of this phenomenon (76,78), full details of the biology of precocious lines were published (79). The essential characteristics are a reduced prepatent time; a reduced reproductive potential, resulting in attenuation of virulence; retention of immunogenicity; and genetically controlled stability of these traits. Due solely to Jeffers's discovery, precocious lines now form the basis of all the commercially available attenuated vaccines, and

others are in development in various countries (163).

Jeffers (Fig. 1B) obtained his B.S. from Cornell University and, like Edgar, completed his Ph.D. at the University of Wisconsin. Unlike many prominent coccidiologists, Jeffers pursued a career in industry (109); during that time he worked with Drs. John Challey and Cornell A. Johnson at Hess and Clark, Inc., Ashland, OH, from 1969 to 1974, then with Drs. Ray F. Shumard and Maury E. Callender at the Lilly Research Laboratories, Greenfield, IN, from where he retired in 2001. Although he suggested in 1974 that precocious parasites would be ideal for an attenuated vaccine (75,76), it is a curious fact that Jeffers never put this idea into practice.

The explanation for this omission is that the patent attorney for Hess and Clark, where Jeffers made his discovery of precocious mutants, believed that such a vaccine would be unpatentable in the light of the prior art provided by the Coccivac® vaccines. (This now seems rather ironic, considering the struggle over prior art that Edgar had with the patent examiners so long before!) Hess and Clark therefore gave Jeffers permission to publish his results (75,76), which automatically prevented anyone else from obtaining a patent on this discovery. Although this would have left Jeffers free to develop an attenuated vaccine when he moved to the Lilly Research Laboratories in 1975, his new company was not interested, being fully occupied with the development of the exciting new ionophorous anticoccidial drugs (T. K. Jeffers, pers. comm.).

It is somewhat puzzling why the opportunity of developing an attenuated vaccine based upon precocious lines was then not immediately taken by other workers in the United States. Before the development of Paracox® was initiated in the United Kingdom, Joyce Keener Johnson (1939–2001) and Prof. W. Malcolm Reid (1910–90) of the University of Georgia at Athens briefly studied, with Jeffers, the immunogenicity of precocious lines of *E. tenella* in floor pens (83), but nothing more came of this work, which was published in 1979. Admittedly, this was the year of Reid's retirement, but Johnson (109) remained to work with his successor, Peter L. Long. With the passing of Joyce Johnson and Malcolm Reid, I have been unable to establish with any certainty why this initial work

with precocious parasites was not continued; neither T. K. Jeffers nor P. L. Long could recall the circumstances. Prof. L. R. McDougald, however, has suggested that lack of funding at the critical time was probably a contributory factor (pers. comm.).

At about the same time, Edgar was successful in producing precocious attenuated lines of *E. tenella* and *E. maxima* (24) and, according to a letter from Edgar to Lasher, he was ready to test them in floor pens in March 1979. Despite this, no precocious lines were ever incorporated in any formulations of Coccivac®. The reason may be that it was then considered uneconomical to manufacture a vaccine containing precocious lines because of the low fecundity associated with them. This concern was mentioned by Edgar in the aforementioned letter, although in his publication (24) he noted that oocyst production of his precocious lines was not much reduced. Hence, the reason for this further failure to utilize precocious parasites in a vaccine also remains a mystery. In passing, might it be that Johnson and Reid at Athens did not continue their own work in this field at this time because of Edgar's paper (24)?

Meanwhile, Dr. Peter L. Long had been working at the HPRS in the United Kingdom on attenuation of coccidia by embryo adaptation until 1979 when he succeeded Reid at the University of Georgia. Long's first few years at Athens were occupied in continuing his embryo work until he and Joyce Johnson turned their attention to producing precocious lines of several *Eimeria* species from American parent strains during the middle to late 1980s (81,82,84,108). This time, the Georgia team continued work on precocious lines with more determination. This was because of the possibility of using them in a beadlet-encapsulated vaccine for trickle infection via the feed. Precocious lines were supplied by the University of Georgia to the Unilever Research team, which developed the beadlet technology (120), and joint work was carried out using the combined resources of Unilever; the University of Georgia; and Merck, Sharp and Dohme (P. L. Long and M. W. Shirley, pers. comms.). Some successful trials had been completed by 1985 (84), but for reasons that are still commercially confidential, this project was terminated without producing a commercial vaccine. A story in circulation during the early 1990s suggested that,

because an in-feed vaccine would come under the authority of the Food and Drug Administration rather than APHIS, the stringent requirements for registration of in-feed anticoccidial drugs had proved to be impossible to achieve with a live vaccine. Indeed, some of the requirements may have been inappropriate.

Despite these various false starts in the United States, Jeffers's discovery of precocious parasites did finally lead to the development of a commercial vaccine in the United Kingdom. During Long's early years at Athens, when he was still working on the attenuation of parasites by embryo adaptation, his former colleagues at the HPRS began in 1981 to develop the precocious parasites that were to become the basis of the Paracox® vaccines.

P. L. Long and embryo-adapted *Eimeria* strains. The discovery of embryo adaptation was due to Dr. Peter L. Long at the HPRS in the United Kingdom. In 1965, he showed that *E. tenella* is able to complete its life cycle in the chorioallantoic membrane of a chicken embryo (102). Subsequently, he found that not only was repeated sequential passaging of parasites through embryos possible (103), but that the parasite line eventually became attenuated (104,106,107). It is due to the work of Long that this alternative method of attenuation was subsequently available for use in the commercial vaccine, Livacox®.

Long (Fig. 1C) began his career in 1949 at the HPRS, where he was technical assistant to Dr. Clifford Horton-Smith. Working later with Dr. M. Elaine Rose and the late Dr. Alan E. Pierce, he gained the degrees of Ph.D. and D.Sc. from Brunel University and became Head of Parasitology in 1972 (109). In 1979, he succeeded Prof. W. M. Reid at the University of Georgia, becoming the D. W. Brooks Distinguished Professor in 1983. He retired in 1989 and returned to the United Kingdom.

A major reason that embryo adaptation has not been more widely used to produce attenuated vaccines is that *E. acervulina*, *E. maxima*, and *E. praecox* are not able to complete their life cycles in embryos (142). Nevertheless, the combination of an embryo-adapted line of *E. tenella* with precocious lines of other species in the Livacox® vaccines has been commercially successful (141).

LINKING THE PAST TO THE FUTURE

Up to the early 1980s, research had generally indicated that it is impossible to stimulate immunity in chickens against coccidiosis with dead antigen (129). Later, however, hopes for the discovery of a recombinant vaccine were raised by several publications of evidence to the contrary (26,92,116,156). Despite considerable investment in this technology during the last 20 yr or so, we still have not witnessed the emergence of a commercially successful recombinant vaccine against chicken coccidiosis. Again, history repeats itself as the gap between proof of the scientific principle and the production of a commercially viable vaccine proves to be enormous.

Certainly, recombinant vaccines would have advantages over the live ones available today. They would probably be cheaper, dosing by injection would be precise, litter management to facilitate recycling of infections would be unnecessary, and caged birds as well as floor-reared birds could be vaccinated. It will be fascinating to see what kinds of anticoccidial vaccines will have become available over the next 50 yr.

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