The Etiology of Bovine Neonatal Isoerythrolysis

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The first cases of neonatal isoerythrolysis (NI) in cattle were seen in the late 1960s in Australia and the United States, and the first published reports appeared in 1970. It is apparent on reviewing these reports (1,2) that the Australian investigators, Dimmock and Bell, and the American investigators, Dennis, O'Hara, Young and Dorris, were unaware of each other's findings. Yet, they both arrived at the same conclusion, namely, that the disease in cattle was man-made and that it had been caused by the use of homologous blood vaccines.

Although the disease is brought about by blood-group antibodies that are passively acquired from the maternal parent, such antibodies are not normally present, especially in cattle. As all of us know who would attain a degree of success in cattle blood typing, those antibodies are not easy to come by. Most are obtained from isoimmune antisera produced by injecting red blood cells (RBCs) from one cow into another. Procedures vary from one laboratory to another as to quantities of RBCs and routes of injection. In the author's laboratory, where we have developed some 70 specifically different cattle blood-typing reagents (3), the usual procedure is to inject either 50 ml of whole, citrated blood, or 50 ml of a 50% suspension of RBCs washed in saline (0.91% NaCl), at weekly intervals, withdrawing serum samples from the recipients just prior to each injection in order to monitor the immune response. Most often we use the I.V. route, but occasionally we inject S.C.

There is, of course, tremendous individual variation between animals with respect to the isoimmune response and there are also marked differences between the blood factors (isoantigenic determinants on the RBC membrane) with respect to their capacity to elicit blood-typing or blood-grouping antibodies. Sometimes a single injection is adequate, but in other instances the recipients remain completely refractory after a series of 3 or 4 weekly injections. In the latter instances we sometimes have success by waiting 2 to 6 months and then administering a single booster injection. Sometimes the blood-group antibodies drop rapidly in titer within a week or two after they make their appearance and the fall in titer cannot be reversed by continuing the weekly injections. In other instances they may level off and persist for months and years thereafter, but this rarely occurs unless the animals have had one or more booster injections. By analogy, and with reference to the same disease in man, it is a well-established fact that some mothers are particularly prone to produce blood-typing antibodies (e.g., those of specificity Rh) when transplacentally isoimmunized by RBCs from the fetus, whereas others remain refractory even though exposed to blood-group antigens one pregnancy after another.

I mention these peculiarities regarding the isoimmune response in order to set the stage for our discussion of bovine NI induced by the use of blood vaccines and as more recently induced, experimentally, by isoimmunizing cows with RBCs from the bulls to which they were bred (4). Experimental production of the disease in cattle (4) was particularly pertinent in view of the earlier failures (5,6) which left doubt in the minds of some whether the disease could be induced in that manner.

The Babesiosis or Tick-Fever Vaccine

The babesiosis vaccine was developed primarily for use in reducing the serious problem of tick fever in Queensland, Australia. It was initially prepared by mixing 0.5 ml of blood from splenectomized calves infected with *B. argentina* with 4.5 ml of blood from an uninfected steer or steers (7). It was said to contain approximately 10^7 organisms. The vaccine was polyvalent in the sense that it contained RBCs from at least two animals, an infected calf and one steer.

The recommended initial dose of the vaccine was 5 ml to be followed indefinitely thereafter by 2 ml doses administered every four months. As I have pointed out elsewhere (8), this would appear to be an ideal regimen for the production and maintenance of blood-group antibodies of unusually high titers, especially when one considers that the vaccine was polyvalent. Moreover, it now seems highly probable that RBCs infected with such organisms as *B. argentina* and *A. marginale* function much more effectively as isoantigens than those from uninfected cattle.

The tick-fever vaccine was first used in 1965 and rather intensively in Queensland. The initial report pointing to it as the causative isoimmunogen was soon confirmed by Langford, *et al.* (9), in 1971. In contrast with the American reports (2,10), the Australian investigators fortified their case histories with blood-

group data on the dams and sires of the affected calves, as well as identification of the blood-group specificities of the major offending isoantibodies (e.g., anti-E' of the complex B system and anti-V of the F-V system). It was apparent that a multiplicity of blood-group antibodies, all of the isoimmune variety, were capable of producing the disease in cattle.

Incidence and Current Status of Bovine NI in Australia

There were no published data relating to the incidence of NI and mortality in the calves of cows vaccinated with the tick-fever vaccine.

In a personal note which I received from Dimmock in June of 1972 she wrote, "In 1968-69 some herds here in Queensland were vaccinated at 4-month intervals because of the tick problem. Haemolytic disease (or NI) occurred on many of the properties and a high percentage of animals had very high titers involving a multiplicity of (blood-group) antibodies."

Dimmock stated that a single 2 ml dose of the vaccine produced blood-group antibody titers of up to 128 in some animals within 3 weeks. These antibody levels fell off rapidly after a single vaccination, but with each subsequent vaccination they persisted for increasingly long periods, e.g., 7 months after vaccination was discontinued, antibodies were detected in 80% of a group of animals that had received more than 6 vaccinations. Some of the antibodies persisted for a year or more after vaccination was discontinued. Thus it was evident from what Dimmock wrote that some of the cows would still have problems with NI calves years after the last vaccination.

In that same note, Dimmock pointed out that the vaccine in use in Queensland up to 1971 has now been modified so that its RBC content is much less and it is hoped, she said, that this and less frequent vaccination will reduce the incidence of animals with persistent high titers.

In 1975, the author had the opportunity to visit Dimmock's laboratory and also the institution where the vaccine is prepared. From what I was able to gather, NI is no longer the problem it was in Queensland calves in the late 1960s and early 1970s.

The Anaplasmosis Vaccine

Dennis and co-workers (2) traced all cases of bovine NI which they observed in Texas to the use of an anaplasmosis vaccine. The same is true respecting subsequent reports of the disease by Mitchel and Morgan and by Kerr, *et al.* (10). However, the manufacturer of the anaplasmosis vaccine (trade name Anaplaz) countered (11) to the effect that numerous biologicals other than, or in addition to, Anaplaz contain bovine blood antigens. The manufacturer asserted that such biologicals as feedstuffs, hormones, drugs, parasites and other vaccines (e.g., lepto) contain antigens which can stimulate the production of antibodies in cows negative for the respective antigen. Although that same view is still being maintained (12), not a whit of evidence has been published to the effect that biologicals other than blood vaccines are capable of eliciting isoimmune hemolysins in titers sufficiently high to produce the disease.

The manufacturer would also make a case for naturally-occurring isoantibodies. However, as pointed out elsewhere (8,13), the only natural antibodies which occur regularly in cattle are those of specificity anti-J. And anti-J cannot be involved in the disease for reasons which I set forth as long ago as 1949.

The manufacturer also inferred that the disease in cattle, like that in man and horse, could be brought about by transplacental isoimmunization. While this may happen in very rare cases (none of which has ever been reported in cattle), it could not account for the rash of cases that suddenly began to make their appearance in Australian and American cattle in the late 1960s. While there is now some evidence that transplacental isoimmunization does occur in pigs (14), Roberts pointed out in his review (15) that there was not a case of hemolytic disease in newborn pigs in Britain which could not be traced to the use of the hog cholera or swine fever vaccine, another homologous blood vaccine which was used extensively in the late 1940s and throughout the 1950s.

The Nature of the Anaplasmosis Vaccine

The carton containing the vaccine reads only that it is "killed" and of "bovine origin." The leaflet contained within the carton states that Anaplaz is composed of *A. marginale* organisms which have been inactivated, concentrated and lyophilized. The leaflet also states that a specially-prepared adjuvant is used for rehydration to facilitate absorption of the vaccine at a proper rate so as to elicit the maximum antigenic response.

There is little in the literature on how the vaccine is prepared. It is generally known that it is prepared from the RBCs of cattle infected with *A. marginale* and that the blood is collected at the height of parasitemia. Brock and co-workers who apparently developed the general method of preparation (16) stated only that the purified antigen was prepared from anaplasma infected bovine blood by several steps designed to remove most of the (blood) plasma and cellular material. Searl (17) stated that Anaplaz is produced by harvesting blood from infected animals at the height of parasitemia. The blood cells are then subjected to washing, lysing, purification and standardization as to dosage. It is then lyophilized, resulting in a desiccated cake which can then be reconstituted with a diluent which serves as an adjuvant. Although not published, it is also known that lysis of the blood cells is accomplished by sonification. If there was any thought that sonification of the RBCs followed by differential contrifugation to concentrate the anaplasma and free them of plasma and cellular debris would suffice to eliminate the RBC antigens, it was misconceived from the start. The electron microscopy studies of Scott and co-workers (18), which were published before the vaccine was

developed, had indicated that the membrane surrounding the small units which comprise the anaplasma and the anaplasma themselves is part of the erythrocyte rather than the infective agent, a view reinforced by the studies of Ristic and co-workers (19).

Whatever the contribution of RBC membranes to the constitution or structure of *A. marginale*, we know from the studies of the author (20) and those of Hines, *et al.* (21), that the anaplasmosis vaccine functions as a most powerful isoimmunogen.

Further Notes on the Case Against Anaplaz

According to the leaflet which accompanies the vaccine, for primary immunization, it is important to vaccinate cattle twice at not less than 4-week intervals. For convenience, the leaflet reads, the two primary doses (each 2 ml) may be spaced up to 19 weeks apart. It also states that a 2 cc. booster dose should be given the following year. However, in our studies (20) we observed that the second "primary" dose, which we began administering 16 weeks after the initial dose, served very effectively as a "booster" dose. For example, serum titers of blood-group antibodies as high as 1,024 were reached as a result of the first primary inoculation after which they soon declined to lower levels (e.g., 64 to 128) or had completely waned by the time the second primer was administered. Then they rose sharply and rapidly in most instances to much higher levels, after which they usually declined to levels in the range of 32 to 1,024 and persisted month after month. Colostral titers as high as 32,768 were common. We had never obtained antisera with such potent blood-group antibodies in years of efforts in producing isoimmune antisera by inoculating cows with RBCs from uninfected cattle.

Hines, *et al.* (21), compared the isoimmune effects of the anaplasmosis vaccine not only with mixed lepto-vibrio bacterins, but also with a vaccine said to be prepared in the same manner as Anaplaz excepting that the RBCs were obtained from uninfected cattle. It was only the Anaplaz vaccine and the *A. marginale*-free RBC vaccine that elicited any detectable serum hemolytic titers. However, in that capacity Anaplaz proved to be far more effective than the vaccine prepared from the uninfected RBCs.

Although the anaplasmosis vaccine regularly gives rise to blood-group antibodies of a variety of specificities (e.g., anti-A, anti-C, anti-F, anti-H, anti-L, anti-S, anti-V, anti-W, anti-Z and anti-H') those of specificities anti-A, anti-F and anti-V are most certain to arise in any recipient that lacks one or another of the corresponding blood factors (A, F and V). Moreover, the titers of anti-A, anti-F and anti-V generally exceed those of any other blood-group antibodies engendered by the vaccine by several two-fold dilutions, and it is usually antibodies of specificity anti-A, anti-F and anti-V which the vaccinated animals continue to make for years after the last inoculation.

From our studies of the isoimmune response to the anaplasmosis vaccine not only in the experimentally inoculated animals but also in numerous animals in private herds in the anaplasmosis endemic areas of California, Idaho, Montana, Nevada and Wyoming, we invariably encounter isoimmune hemolysins of specificity anti-A, anti-F and anti-V. This is the hallmark of Anaplaz as an isoimmunogen and it is the hallmark which readily distinguishes that isoimmunogen from all others, including the tick-fever vaccine.

Although there is no published information on the numbers of animals or the breeds of animals whose blood is used in the preparation of each lot of the vaccine, it is our considered opinion that 40 to 50 animals are used and that they are predominantly of the Holstein-Friesian breed. This opinion is based on several factors all taken into consideration in assaying the quality and quantity of the isoimmune response. Those factors include a knowledge of the frequencies of the genes which control the blood groups (particularly those in the A and F-V systems), a knowledge of the comparative isoantigenicity of the various cattle blood factors (e.g., A > V > F), and the economics of vaccine production.

All things considered, especially the fact that the anaplasmosis vaccine is a most powerful isoimmunogen, the case against Anaplaz as the isoimmunogen responsible for NI in the U.S. calves and, more recently, in Mexican-born calves (22) is a particularly strong one.

Diagnostic and Prognostic Tests

Elsewhere (8) I have described the various serologic tests which are used to affirm or deny clinical diagnoses of bovine NI. The simplest test of all is to prepare a 2½ to 3% suspension of saline-washed RBCs of the affected calf and to 0.10 ml of the suspended RBCs one adds an equal quantity of fresh rabbit serum which serves as the source of complement in all cattle blood-typing tests. If the RBCs are sensitized with blood-group antibodies, they will become hemolysed within one hour at temperatures in the range of 23 to 30°C. If the RBCs are not sensitized, they will settle to the bottom of the tube. There may be a tinge of hemoglobin in the supernate above the button of cells as the RBCs of most newborn calves are slightly labile in rabbit serum.

The test can also be performed on blood withdrawn from dead calves just as long as the RBCs remain stable in the saline solution (0.91% NaCl). It should be noted that rabbit complement is available commercially through Pel Freez Biologicals, Inc., Rogers, Arkansas.

The most convincing diagnostic tests are the antibody-elution tests. If the RBCs of the calf are sensitized, some of the blood-group antibodies can be heat-eluted from the cells at temperatures in the range of 50 to 56°C. In our laboratory we test the eluate against a panel of RBCs from cattle of known blood groups to determine the specificity of the eluted

antibodies. Generally, they are of specificity anti-A, anti-F and anti-V. But we have also been able to elute antibodies of other specificities (e.g., anti-H, anti-L and anti-H'). Sometimes the eluate contains antibodies of such a variety of specificities that it is difficult with the limited amount of fluid to determine their specificities.

Also diagnostic of man-made NI is the fact that the serum of affected calves often contains unbound blood-group antibodies. For the most part, such unbound antibodies are of specificities other than those which are present in the eluates. This is as expected because most often the affected calves do not inherit from their sires all the blood factors corresponding to the isoantibodies demonstrable in the serum or colostrum of their dams. For example, if the dam's serum or colostrum contains anti-A and anti-V, and the calf inherited V and not A from its sire, anti-A remains unbound in the calf's serum whereas anti-V, if not present in excess, is found only in the RBC eluate. Such observations make it clear that the anti-A could not possibly have been engendered as a result of transplacental isoimmunization of the dam by RBCs from the fetus. And it is such observations that distinguish these man-made cases of NI from the naturally-occurring cases seen in horses and man.

Any antibodies eluted from the RBCs of affected calves will react with the RBCs of their respective sires but most often blood samples from the sires were not available at the time the tests were performed.

The object of the prognostic tests is to determine whether the serum of pregnant cows contains isoimmune antibodies that might react with the RBCs of the forthcoming calves. This requires blood samples collected from the cows any time during the last trimester and also blood samples from the bulls to which they were bred, in the event such bulls do not already have their blood types on record. If the sire has one or more blood factors corresponding to isoimmune hemolysins demostrable in the serum of the cow, the forthcoming calf is considered at risk, especially if the serum titer of the antibodies is greater than 32.

Prognosis is illustrated for blood factors A, F and V in Table 1. Blood factors F and V are inherited as allelic alternatives, whereas A is inherited independently of F and V (3). There are, therefore, six possible A-F-V blood types and their occurrence is determined by the frequencies of the genes which code for the respective determinants in the particular breed under study.

We show in the left column of Table 1 the six A-F-V types of anaplasmosis-vaccinated cows and all of those types will usually be represented in a herd of 100 or more brood cows. However, the incidence of the six types often varies significantly between herds and breeds. In the second column of Table 1 we note the A-F-V antibodies present in the serum of the vaccinated cows. In the third and fourth columns of Table 1 we indicate, respectively, the A-F-V blood types of forthcoming calves that would and would not be at risk, taking into account that a cow of any A-F-V type can be bred to a bull of any A-F-V type.

The reader can satisfy himself or herself that none of the calves of type F cows would be at risk to anti-A and anti-V if the sires of the calves are all of type F, because all of the calves from F x F matings are of type F. On the other hand, 50% of the calves sired by bulls of type FV out of type F cows would be at risk to anti-V because, on the average, 50% of those calves will inherit V rather than F from the sire. However, if all of the sires are of type V then all of the calves would be of type FV and all would be at risk to anti-V. In considering the A factor, at least 50% of the calves sired by a bull possessing that factor and out of cows lacking that factor will inherit A from the sire and will be at risk to anti-A. The figure is 100% if the bull happens to be homozygous for the A factor. Thus, the incidence of NI in one vaccinated herd can vary significantly from that in another merely by chance selection of breeding bulls. Moreover, the incidence in one herd can vary significantly from one calving season to the next, depending upon chance rotation of breeding bulls.

In our laboratory we performed prognostic tests on an experimental basis in a rather sizable herd of registered Herefords over two calving seasons, taking

*Table 1: The A-F-V blood types of cows; the antibodies of specificities anti-A, anti-F and anti-V which they produce following inoculations with the anaplasmosis vaccine; and the possible A-F-V blood types of their forthcoming calves, in matings to bulls of all A-F-V types, showing the types of the calves which would be and would not be at risk to the passively acquired antibodies.

A-F-V blood types of cows	Antibodies engendered	Possible blood types of calves showing those	
		At risk	Not at risk
F	anti-A & anti-V	AFV, AF, FV	F
V	anti-A & anti-F	AFV, AV, FV	V
FV	anti-A	AFV, AF, AV	F, V, FV
AF	anti-V	AFV, FV	AF, F
AV	anti-F	AFV, FV	AV, V
AFV	none	all 6 types but none at risk	

*This table considers prognoses involving only the major isoimmune hemolysins engendered by the vaccine. There are others (see text).

into consideration not only A, F and V, and their corresponding antibodies, but other blood factors such as C, H, L, S, W, Z and H'. When the prognosis was poor the owner removed the calf from its dam and fed it on colostrum and milk from non-vaccinated dairy cows for the first two days, after which the calf could be safely returned to its dam. If the prognosis was good, the calf was left with its dam. The results were most successful, excepting in one case where the owner switched a calf with good prognosis to a foster cow that had strong anti-A in her colostrum. The calf, which could have inherited the A factor from either or both of its parents, became severely afflicted with NI, but recovered.

Bovine practitioners continue to be amazed when they encounter NI calves in herds in which the cows received their last booster inoculations over two years prior to the birth of affected calves. Naturally, some of them doubt their own diagnoses. In one instance we confirmed a diagnosis of NI in which the cow had last been vaccinated with the anaplasmosis vaccine 7 years prior to the birth of the affected calf. The cow was still making anti-A and it was possible to elute anti-A from the sensitized RBCs of the calf.

Experimental Cases of Bovine NI

Theoretically, it should be possible to produce NI or equivalent isohemolytic syndromes in virtually any vertebrate species in which naturally-occurring cases of the disease must be very rare if they occur at all. A cogent example was the production of NI in the domestic chicken (see references 8 and 13).

The best way of producing the disease experimentally, barring the use of blood vaccines, is to repeatedly inoculate the mothers-to-be with RBCs from the males to which they are to be bred and to continue administering booster injections when the females become pregnant. Alternatively, one can use RBCs from animals other than the breeding males, but the RBCs must bear some antigenic determinants common to the breeding males but not to the females.

Where some investigators have failed to produce the disease, others have succeeded. This was true not only in the experiments using rabbits, but is now true of those in which cattle were used as the experimental animals. Until recently, it appeared from the earlier experiments of Braend (5) and Kiddy, *et al.* (6), that it might be difficult, if not impossible, to produce NI in calves by isoimmunizing cows with RBCs from the bulls to which they were bred or by utilizing RBCs from other animals sharing some blood factors with the bulls. However, in all fairness to Braend, it appears that his main interests were to determine whether there was any passage of the isoantibodies from mother to fetus, rather than to induce the disease experimentally.

In 1973, Kerr and co-investigators (23) reported that they produced NI in calves from dams that were injected with washed, lysed RBCs from the bull to which the cows were bred. Each injection consisted of 15 ml of packed lysed RBCs plus 10 ml of adjuvant and each cow was given 3 injections. Their abstract contained no information relating to the blood groups of the animals of the specificity of the offending isoantibodies.

In contrast, Dimmock and colleagues (4) have shown conclusively that it is possible to produce the disease experimentally in cattle and they were able to identify the major isoimmune hemolysins that were responsible for the disease. Those antibodies were found to be reactive with blood factors, B, G, O, T, Y and G' of the B system, C of the C system, F and V of the F-V system, L of the L system, S, U and H' of the S system, and Z of the Z system. Among the 15 affected calves (sired by one or the other of two bulls) out of six cows over a period of from 1 to 4 gestations, two died, one with a peracute case and the other with a chronic case. The remaining 13 managed to survive with days to recovery ranging from 10 to 56. Generally, the severity of the disease was correlated with the titer of the antibodies in the colostrum. Cow number 6, the dam of the two calves that died, maintained a high level of antibody for over two years after the last inoculation. (Each cow received multiple inoculations of washed, packed RBCs. The amount per injection was 25 ml and, with one exception, the inoculum was from the bull to which each cow was bred.) In the peracute case, the cow had received her last injection 52 weeks prior to the birth of the calf.

I mention these features here mainly because they relate in many ways to what we see in the cases caused by the use of blood vaccines. The same is true with respect to the histopathologic features of the disease, the hematologic findings, etc., which are not reviewed in this report because they are so well documented in the reports of Dimmock and co-workers (1,4) and the initial report by Dennis and co-workers (2).

Responsibilities and Recommendations

Homologous blood vaccines have taken a heavy toll in the lives of newborn calves (8). They have also been responsible for the deaths of many piglets (15) and perhaps some foals as well (8,15). In view of the history of such vaccines, it is an obvious risk to use them on breeding females. Once they are on the market it is the practicing veterinarian who will usually be faced with the final decisions concerning their use. He or she must weigh the protective immunological effects of the vaccines against side effects such as NI. In any event, the veterinarian cannot be held at fault, especially in view of the inadequate warnings (or none at all) that accompany the vaccines.

Where, then, does the responsibility lay – with the licensee or the licensor, or both? In an editorial by A.M. Prince, which appeared in the 25 March 1977 issue of *Science*, this very question was discussed but not with reference to blood vaccines. Rather, his editorial was concerned with recent mass swine influenza immunization program, a program decided upon and underwritten by the U.S. Government. As is

well known, there were some deaths associated with the use of the influenza vaccine. Whether those deaths were wholly coincidental or were somehow related to the vaccine may never be known. As Prince points out, in the litigious climate that exists in the U.S., these events will inevitably result in lawsuits.

He suggested two possibilities: One would have the government undertake to bear legal responsibility for all products that it has licensed, and therefore (presumably) tested and approved, unless negligence in manufacture or administration can be proved. The second would have the government itself, or nonprofit government-supported organizations, take over the manufacture and distribution of biologics, as widely done in countries such as Sweden and France.

But Prince was only debating the issues as related to biologics manufactured for human use and not those manufactured for animal use. Nevertheless, the principles are the same and, in any event, man suffers whether the damage effected by such products is to his relatives or to his livestock.

I take the position that any government which licenses a blood vaccine should be equally responsible, if not more so, than the licensee or manufacturer, especially when the vaccine has not been properly monitored for side effects such as NI. There is no evidence, as far as I am aware, that the question of such side effects was ever raised in the development of the anaplasmosis vaccine. Moreover, there is no indication that the U.S. Government has done anything more than duck the issue. What, then, can be done?

For one thing, the NI problem could perhaps be lessened considerably by limiting the manufacture of the anaplasmosis vaccine to the use of RBCs from cattle of selected blood types. For example, if each lot of the vaccine were produced only from cattle of type F of the 6 A-F-V types shown in Table 1, this would eliminate the production of anti-A and anti-V in the vaccinated cows. The only major antibodies that would still be a problem would be those of specificity anti-F. Although cows of type V would produce anti-F, animals of that type are usually less frequent than F and FV in most of our cattle breeds.

Another approach would be to produce the vaccine using *A. marginale* infected RBCs from other species which share few or no cross-reactive blood factors with domestic cattle. However, if the protective effects of the blood vaccines are associated with the isoantibodies they elicit, such measures would be to no avail excepting that the calves might all survive. Whether the protective effects of the blood vaccines might in some way be related to the qualities and quantities of the isoantibodies they elicit is yet to be investigated. It is possible that the C.F. antibodies are closely correlated with the blood-group antibodies, not only with respect to titer, but also with respect to persistence and specificity.

Finally, a great deal of caution should be exercised when it comes to vaccinating brood cows and replacement heifers. One thing not to be recommended is booster injections.

Eventually, such organisms as *A. marginale* and *B. argentina* may be propagated in tissue cultures, or may be multiplied outside of RBCs. When that day comes it may be possible to prepare useful vaccines that do not have the capacity to engender isoantibodies.

Summary

In this article I have devoted attention mainly to the use of blood vaccines that are capable of eliciting blood group antibodies in cattle and can by themselves account for all cases of NI in calves other than those experimentally produced. The vaccines at fault have been identified as a tick-fever vaccine used in Queensland, Australia, and an anaplasmosis vaccine used in the U.S. and marketed under the trade name Anaplaz. Both vaccines have been shown to be powerful isoimmunogens which, when used as recommended, give rise to isohemolysins which continue to be produced in some cows years after the last inoculation.

While there are some who still contend that biologics other than or in addition to Anaplaz could be responsible for the field cases of NI in American calves, evidence to that effect has not been forthcoming. There are also some who claim that NI has been diagnosed in American herds in which the cows were never vaccinated with the anaplasmosis vaccine. However, as far as I am aware, there is no evidence that such field diagnoses were ever confirmed by appropriate serologic tests. On the other hand, it is known that some of the initial field cases of NI in American calves were confused with anaplasmosis itself. Both NI and anaplasmosis are primarily characterized as anemias. In that connection, it is of historical interest to note that a vaccine produced to alleviate one anemia induced another.

But there are other side effects, as it were, which were not touched upon, largely because they have not been documented in the literature and some of them are not likely to be.

It is customary in veterinary practice that when a cow needs a blood transfusion one draws blood from the nearest healthy donor and proceeds with the transfusion, knowing very well, as taught in veterinary manuals and elsewhere, that a first transfusion can usually be performed without fear of transfusion reactions. That is no longer true, because many of the Anaplaz and tick-fever vaccinated animals have isoimmune hemolysins often in quite high titers. I know of only one accident of this nature, but suspect there have been more. The veterinarian unknowingly drew the blood for the fatal transfusion from an Anaplaz-vaccinated cow.

The question may be asked, "Do the calves which survive NI have any lingering effects of that disease?" Probably so, and apparently there is now unpublished evidence that such calves behave as if they are splenectomized, or partially so, because they are reputed to be very susceptible to anaplasmosis and develop the acute symptoms of the disease as seen in

adults. These and other possible side effects of the vaccine are in need of further study.

But where, do we ask, does one get the funds? Hopefully, there will be funds available for promising young research workers to spend careers in search of the answers to the many problems posed by hemotropic diseases such as anaplasmosis, babesiosis, trypanosomiasis, and the theilerias as well as the problems imposed by the vaccines. In the meantime, blood-group workers will just keep plugging along and possibly some of us will still be around to help solve the problems raised, not only by the aforementioned diseases, but those that might be raised as a result of the vaccines used to combat them.

My advice for the "young-uns" who would aspire to enter these areas of research is to live and breathe genetics, especially the dynamic areas opened by that branch of genetics sometimes referred to as immunogenetics. The latter is the discipline from which all knowledge of animal blood groups has arisen, and it was cattle blood groups which were the first to be thoroughly explored.

While such blood-group knowledge was applied effectively in the diagnosis of NI and recognition of the etiological agent responsible for the NI in Australian calves, the same could not be said about the American cases, at least until recently. This is perhaps the main reason why it was possible to promulgate so much doubt concerning the etiology of the American cases. I think the air is now clearing.

Acknowledgement

Naturally, I no longer perform the serologic tests mentioned in this report. These are handled by B.G. Morris and Y. Suzuki, and I am grateful to them. I am also grateful to Drs. Barry Duelke and Maarten Drost for the inoculation of the cows in our experiments designed to monitor the isoimmunologic properties of the Anaplaz vaccine, and to Dr. J.C. Trace, of Fort Dodge Laboratories, for providing one experimental lot of the vaccine. I am also indebted to Prof. R.C. Laben for use of dairy cows in the U.C. dairy herd for the experiments. Then there were many veterinarians who submitted blood samples to our laboratory for NI diagnoses and prognoses. Their comments and the samples which they forwarded were most helpful for rounding out details concerning the etiology of the disease in cattle of the western states, and also through contact with these veterinarians I was able to learn more about the prevalence of the disease and other problems related to it.

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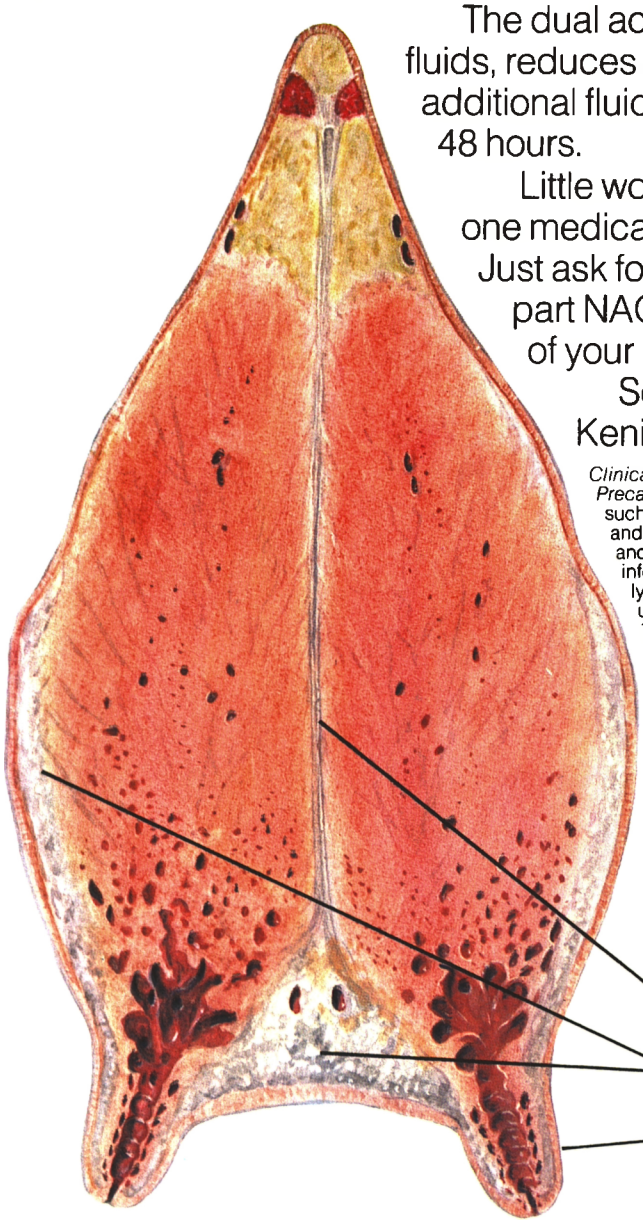

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