It is significant that most countries have evolved distinct types of stock to suit their conditions and in time we would expect much the same thing to occur in this Dominion, and possibly economic pressure in the future may tend to speed up such development.

Commenting on the query regarding grazing management of cocksfoot-Italian sowings in their early stages, Mr. Levy advised the grazing down of the Italian ryegrass at the 4" to 6" stage before any smother of the smaller cocksfoot plants took place.

The Italian was added for the purpose of controlling weeds and of making possible earlier grazing than was possible when cocksfoot white clover alone is sown.

"ON VACCINE TREATMENT FOR MASTITIS."

by

M. E. Marples.

In a recent address to the Royal Society of Arts, in London, Dr. R. O. Tisdall(1), speaking of M. & B.693, made the following remarks on new treatments in general:-

> "One gets the impression that there is a definite swing of the pendulum. First, the notorious conservatism of the British medical profession, doubting gravely as to its having any value at all; then, the insistent enthusiasm of those who have tried it and found it entirely satisfactory, followed immediately by the equally insistent pessimism of those who have tried it and found it entirely useless; and, finally, the general acceptance of its proper position in the Materia Medica according to the equally British faculty for compromise."

These remarks may, I think, be fairly applied to the position of mastitis vaccines, which are now in the second phase. I submit that a frank discussion will enable them to approach the third stage. A review of their present position will allow us to appreciate their possibilities and to acknowledge their limitations.

Many of those who have tried mastitis vaccines have their full share of the "insistent pessimism" referred to, but it can hardly be denied that there have been instances of their successful use. For instance, C.S. Bryan(2) tried autogenous killed and autogenous live vaccines, using injections every week, and claims to have reduced considerably the number of infected quarters.

Otto Stader(3), speaking at the 12th Annual Veterinary Conference in 1934, reported excellent results on the prevention of mastitis from the use of 4 or 5 doses of autogenous vaccines made up from all strains occurring in normal udders in a small herd.

Seddon & Rose (4) used an autogenous vaccine on a herd of 85 cows, and claim that, although the incidence was not altered, the percentage of clinically recognizable cases, the severity of the cases and the liability to recurrence, were all lessened.

And within the last two months, come reports from England, where G.N. Gould (5) and H. M. Wilson (6) used a staph. toxoid vaccine on cases of sub-acute staph. mastitis. Gould

says the treatment "exceeded expectations." Wilson gives fuller details regarding 4 cows whose milk was found to contain staph. germs. The cows were given doses of 10, 15 and 20 ccs. at intervals of 3 days, and the milk, 14 days later, was found to be free of infection. In these two cases, the milk examinations and the preparation of the vaccine were carried out at the Wellcome labs. at Beckenham, and the results should, therefore, be above suspicion.

On the other hand, Plastridge et al (7) used heat killed autogenous vaccines at intervals of 5 weeks to 3 months, over periods of from 2 to 6 years. They report no appreciable difference in incidence of mastitis between vaccinated and control cows.

Dr. Hucker, replying to a question at a meeting in Hamilton, stated that, as far as his experience of American vaccines went, the results were negative.

More emphatic statements than these have been made. Udall (8) states, "there is no evidence that vaccination exerts either a curative or a prophylactic effect."

Seeleman, of Kiel (9) says that vaccination, both prophylactic and curative, is useless.

These two emphatic statements are rather unfortunate, in that they give the impression that vaccination is a standardised procedure, and that vaccines are standard preparations like, say, baking soda. Both these impressions would be quite incorrect.

Gaiger and Davis (10) sum up the position in one sentence, "Vaccines used for curative purposes, or in an endeavour to limit the spread of infection in a herd, have given very variable results."

But the question then arises "why these variable results?" If some cases can be cured, why not the majority? There is no need to stress the economic importance of mastitis, and any line of attack, which holds out any hope of reducing the loss, is worth following up. I should, however, point out that results approaching 100% are not to be expected in such a complicated disease as mastitis. 100% cures or absolute immunity are not obtained even in the most clear-cut infections due to single organisms. But I believe that, if we can find out the reasons for the different results reported, we may have taken some steps towards solving the problem. I will give four possible reasons for these variations, the larger part of which must, I think, be due to the different vaccines used.

First of all, there is the question of the concentration of the suspension of bacteria in the vaccine. This point seems to have been neglected by most workers, and, among all the papers and abstracts to which I have had access, only one, that by Seddon and Rose, mentions the concentration of vaccine used. Theirs contained 4,000 mlns, per cc. The usual instructions on a bottle of vaccine refer to the volume of the dose to be given, usually 1 or 2 ccs., but it is obvious that the results to be expected will vary according to the concentration of the suspension, i.e. according to the total quantity of dead bacteria.

I have only been able to examine a few samples of various vaccines on the market, but, even in these few, the differences of concentration are surprisingly large. For instance, here is a sample of an American vaccine which contains less than 25 mlns. per cc. This second sample of a New Zealand vaccine contains over 2,500 mlns, per c.c.; it is more than 100 times as concentrated. In between comes an English vaccine with about 2,000 mlns. per cc. The label states 20,000 mlns! This sample is very impure, and nearly

half of its turbidity is due to extraneous matter of some sort.

The second reason, closely allied to the concentration, refers to dosage, and on this point also, very few of the original papers give details. Seddon and Rose, who also gave the concentration at 4,000 mlns., used 2 doses of 10 ccs. each, at an interval of one week. The recent notes by Gould and by Wilson state that 3 doses were given, viz., 10, 15, and 20 ccs. It is unfortunate that these two workers do not give the concentration of the vaccine used, especially as they claim such excellent results. Of the vaccines I have mentioned, the American dosage recommended is 2 ccs., the English one recommends 0.5 cc. and 1 cc. for prophylaxis purposes, and 0.25, 0.5, and 1 cc. for curative purposes. The New Zealand preparation recommends four doses, 5, 5, 5 and 10 ccs. for first inoculations of the whole herd, and for curative purposes, 20, 30 and 40 ccs. or 30, 40 and 50 ccs. Doses of 100 ccs. and even higher have been given in long established, obstinate cases. Working out these dosages in conjunction with the concentrations, we find the American prophylactic dose is less than 50 mlns. of bacteria, the English doses are 1,000 and 2,000 mlns. for prophylaxis; 500, 1,000 and 2,000 mlns. for cure; while the New Zealand doses are 3 at 12,500 and one at 25,000 mlns. for first injections of the whole herd, while curative doses mount to 125,000 mlns. and even 250,000 mlns. or more for obstinate cases. Different results might reasonably be expected from dosages which vary to that extent.

The New Zealand doses may appear, at first sight, to be unduly large, but a comparison of these doses with doses given to human beings, show that they are not excessive. Body weight is recognised as being an important item in dosages. In working on the standardization of toxin-antitoxin mixtures, the Minimal Lethal Dose is defined as "the least amount of toxin that will, on the average, kill a guinea-pig OF 250 Gms. WEIGHT, within 96 hours.(11) In human beings, the prophylactic doses of say typhoid vaccine are usually 1,600 and 3,200 mlns. or roughly 10 and 20 mlns. per lb. of body weight. Cholera prophylactic doses are slightly heavier, 2,000 and 4,000 and sometimes 8,000 mlns.(12) of cholera vibrios, i.e., 12, 25 and 50 mlns. per lb. of body weight. The doses of 12,500 and 25,000 mlns. for this vaccine are roughly in the correct proportions, about 12 and 25 mlns. per lb. for a Jersey cow, and 10 and 20 mlns. per lb. for Frisians. No analogy with human vaccines for curative purposes is possible, as the usual practice with humans is to give doses of antitoxin, instead of vaccine, when the patient is ill.

If this argument in favour of large doses is correct, it is only reasonable to expect no result from doses of 1 or 2 ccs. There may, however, be a reason for the makers' recommendation of small doses. Vaccines, when first prepared, are definitely poisonous or toxic. Von Glahn and Weld (13) have shown that staph. aureus toxins cause lesions and partial necrosis of the kidney. Weld (14) found that strep. toxins also affect the tubular epithelium of the kidney; both experiments being conducted on rabbits. The toxic properties can be removed by chemical means, and the vaccine is then called a toxoid vaccine. If this procedure is carried out, large doses can be given without any harmful effects, such as fever, loss of appetite or of milk, which sometimes follow the injection of even small doses of untreated vaccines. The removal of the toxic properties from a vaccine does not, as a rule, affect its antigenic value, i.e. its power to produce antibodies in the system.

The first two possible reasons for variation in results are quantitative. The third is qualitative and, in my opinion, is the most important. It was established many years ago that each particular disease requires its own vaccine, that is to say, vaccines of killed bacteria are only efficacious when used to combat disease caused by the same bacteria. It has also long been known that mastitis can be

caused by a variety of organisms, such as strep. agalactiae, staph. aureus, B. coli, various micrococci, etc. But it has been appreciated only fairly recently that many bacteria, classed under the same name, are of different strains. Streps. seem particularly liable to this variation in strains, and the strep. which causes pneumonia, for instance, is known to have at least 32 strains which are different in their antigenic content.(15) The original strep. agalactiae has now been found to contain strains which are different in some biochemical respects. These are labelled by Minett and others (16), (17), Groups I, II and III. Stableforth (18) has further subdivided each of these groups by serological tests. Thus of Group I, 91 strains could be split up into 61 of serological type I(a), 6 of type I(b), and 24 of type I(c). 29 strains of Group II were divided into 12 of type II(a), 10 of type II(b), and 4 of type II(c). 40 strains of Group III gave 5 of type III(a), one of type III(b), 9 of type III(c), while 25 strains gave a negative result with all serological tests.

As strains of the different biochemical groups were entirely distinct serologically, the conclusion is that what is usually called strep. agalactiae is really one or more of a bunch of at least nine streps, which, from the serological point of view, i.e. the vaccine point of view, can be regarded as different bacteria. A vaccine prepared from a strep. of, say, Group II, type II(a), would have no effect on an infection of a strep of, say, Group I, type I(c). Within the last few years, the list of types has been further extended by Stewart (19), and by Murnane and Clark (20) in Australia.

An appreciation of the wide variety of strains goes a long way to explain the varying and usually negative results obtained by different workers. With such a variety of infection, it may be suggested that better results would be obtained with an autogenous vaccine for each case. This may be true, but it is hardly practicable when dealing with large numbers of cows. It has seemed preferable to prepare a stock vaccine with as large a coverage as possible over all types of udder troubles, and to prepare autogenous vaccines for those cases where the stock vaccine gives no result.

Of the other bacteria known to cause mastitis, B. coli also gives various different serological strains, but as coli is not a major cause of mastitis in New Zealand, the differences are not so important as those occurring in streps.

The fourth possible reason arises from the method of production of the vaccine. There are two methods in use, first, growing the bacteria on a solid medium, such as agar, and washing off the growth with salt solution; second, growing the bacteria in broth. The difference is important. A single bacterium frequently has various antigens. One bacterium may excrete its antigens during growth, another may retain them within the cell substance, in which case they can only exert their influence when the cell disintegrates; a third may possess antigens of each kind. As antigens are soluble in aqueous media, those excreted during growth on agar would be absorbed by the agar and would not be found to any appreciable extent in the suspension after washing off the growth with salt soln. By growing bacteria in broth, and using this broth as vaccine, all antigens, whether excreted or retained, are present in the final product. Streps. are among those bacteria which excrete antigens during growth, though some may also be retained, and for this reason the broth method of culture should be used for strep. vaccines.

The American vaccine previously mentioned has been grown on a solid medium; the English one is probably the same, though there is some doubt about it; the New Zealand one is prepared in broth. The disadvantage of the broth method is that concentration is restricted. As the bacteria grow, they produce acids, and the gradual increase in acidity leads to a cessation of growth and the death of the organism. The

maximum concentration of streps. in the broth used, is about 3,200 mlns. per cc. but other strains are killed at about 2,700 to 2,800 mlns. In order to inject the requisite amount of antigenic material, massive doses are necessary.

Very few of the published papers on mastitis vaccines make any mention of the type of vaccine used, whether washed or broth, and this may be one cause of varying results. There are probably other causes besides the four mentioned, but these, I think, are quite capable of producing widely varying results.

To refer the New Zealand vaccine to these four points. It is prepared in broth; it has as high a concentration as can be expected; and it is so treated that massive doses can be used. The question of strains is much more difficult. We attempt to obviate it by using as many different strains as are available, and usually the vaccine is prepared from 12 to 16 different strains of strep. In an endeavour to cover all types of udder troubles, growths of other bacteria known to be frequent causes of mastitis in New Zealand are also added. I cannot claim that all strains have been covered, as, during the last season, 38 autogenous vaccines were supplied on request. So far, three adverse reports on these vaccines have been received.

Made and used in these ways, this vaccine has achieved a considerable degree of success. Being scientists, you would like some scientific backing for this statement, but also, being scientists, you will appreciate the fact that scientific confirmation is exceedingly difficult to get. Wilson, in his recent note, gives particulars of 4 cows, cured by administration of staph. toxoid. The numbers are small. From the economic point of view, a more practical test would be to follow the mastitis history of a large number of cows, say, 500 or 1,000, through two or three seasons, by means of monthly leucocyte chartings. These figures are not available, as only very few, less than a dozen, of the users of this vaccine send in their samples at all regularly. Nor can I give you an accurate picture of the results achieved by quoting from the small number of milk samples received. Dozens, I might say hundreds, of farmers who renew their contracts year by year, never send in any samples at all. Judging from the fact that they do renew their contracts, it may, I think, be assumed that they are getting good results. Some farmers state that they are getting 100% cures, and do not see the need for monthly charting. As we get so small a proportion of milk samples for examination, it is impossible to give any figures for percentage cures. Any figure I could quote would be an estimate only and inadmissible.

I can, however, give you other figures which, though not having the same scientific value, do carry a certain amount of weight. This vaccine was first produced some nine or ten years ago, and started in a small way. With the exception of one year, 1936, its use has extended steadily year by year. Last season it was supplied under contract to over 500 farmers who used it on over 35,000 cows. This has meant a production of a little over four tons; the production for the previous season was just over three and a half tons.

So far, I have said nothing about immunity. In our experience, there is no immunity against strep. infections that is of any practical value. There is a certain amount of negative evidence in favour of a partial immunity from staph. infection. This slight evidence is in accord with medical findings (21) where doses of staph. toxoid have increased the amount of antitoxin in the blood of humans; the increase lasting in some cases as long as ten months. The proved production of antibodies in the blood would certainly lead one to expect a certain degree of immunity, but whether the antibodies are unstable chemically, whether they are absorbed by organs of the system, such as the liver or the spleen, or

whether they are excreted either with the urine or the milk, I am unable to say. Immunities in human patients, either natural or acquired, vary greatly in their degree and the period during which they last. Scarlet fever and erysipelas are both caused by streptococci (22). One attack of scarlet fever usually gives immunity for life, and a relatively high degree of immunity is conferred by vaccines. Erysipelas, on the other hand, can affect a patient frequently, and one attack gives no immunity whatever. So that the absence of any appreciable degree of immunity from bovine strep. infections has its parallel in human diseases. Until we have more knowledge and possibly until we can improve our present vaccines, it would be unsafe to assume that there is any immunity whatever.

In contrasting the three vaccines, of which you have seen samples, I gave the so-called prophylactic doses of two of them because both the American and English vaccines specify the prophylactic dosage on their labels. I contrasted this dosage with what I called the whole herd inoculation of the New Zealand vaccine. This does not imply that any immunity is given. When a farmer first starts any vaccine, his herd is usually in a pretty bad state. He is aware of most of the clinical cases, but there may be an indeterminate number of sub-clinical cases, or cases of recent infection, of which he is not aware. Consequently, we have found it advisable to dose every cow in the herd with the small doses of five and ten ccs. at intervals of from 4 to 7 days; and, of course, subsequently giving the much larger doses to those cows showing clinical symptoms. The smaller doses to the apparently clean cows may also have an additional advantage. The first injection of a single toxin or a vaccine has usually very slight effect on the production of antibodies in the system of most animals (23). The second injection, which may be of the same amount of toxin as the first, causes rapidly a very large increase in the amount of antitoxin. Usually, the second dose is given after the disappearance of any undesirable symptoms. This may be after some weeks, as in the preparation of diphtheria antitoxin in the horse, or after a few days, in those cases where there have been no undesirable symptoms. But whatever the length of time between the two injections, there is a very large increase in antibody concentration from this secondary stimulus. This is the case with laboratory animals, and it probably occurs in the cow. Apparently, the system takes some time to adjust itself to the formation of antibodies, but the adjustment, once made, is not lost after considerable lapse of time. By these initial injections, the cow is prepared for the prompt production of antibodies, in case of subsequent infection, necessitating massive doses of vaccine.

It is possibly this reaction to secondary stimulus, which accounts for the results claimed by Seddon and Rose, especially the diminution in the severity of cases.

S U M M A R Y.

(1) A brief review of some of the published results on mastitis vaccines.

(2) Four possible reasons which might account for the variable results published.

(3) Steps taken with a New Zealand made vaccine to overcome these difficulties.

(4) Progress in the use of the New Zealand vaccine as a cure.

(5) No evidence of any appreciable degree or period of immunity.

REFERENCES.

1. R.O. Tisdall: J. Roy. Soc. of Arts. 1941. p. 628.
2. C.S. Bryan: J. Am. Vet. Med. Assn. 83:390.
3. Otto Stader: Report 12th Ann. Vet.Conf. Illinois, 1934.
4. Seddon & Rose: Austr. Vet. J. 10.
5. G.N. Gould: Vet. Rec. March 7th, 1942.
6. H.M. Wilson: Vet. Rec. April 4th, 1942.
7. Plastridge et al: Bull. Storrs Agr. Exp.Stn. No. 197.
8. Udall: "Practice of Vet. Medicine." p.600.
9. Selleman: "Strep Infections of the Udder."
10. Gaiger & Davis: "Vet. Path. & Bact." p.184.
11. Topley & Wilson: "Bacteriology & Immunity." p.169.
12. Dudgeon: "Bact.Vaccines & their position in Therapeutics." p. 25.
13. Von Glahn & Weld: J. Exp. Med. 1935. p.1.
14. Weld: J. Exp. Med. 1935. p. 473.
15. Cooper et al: J. Exp. Med. 40:461: 55:331; Am. J. Pub. Hlth 25:469.
16. Minett & Stableforth: J. Dairy Sci. 5:223.
17. " " & Edwards: J. Comp. Path. 42:213; 45:1.
18. Stableforth: J. Comp. Path. 45:185.
19. Stewart: J. Path.Bact. 45:279, and Austr. Vet. J. 14:180.
20. Murnane & Clark: Austr. C.S. & I.R. Bull. 134.
21. Fleming & Petrie: "Recent Advances in Vaccine & Serum Therapy", p. 374.
22. Topley & Wilson: "Bact. and Immunity." p. 1170.
23. Fleming & Petrie: "Recent Advances, etc." p. 424.

DISCUSSION.

DR. C.M.S. HOPKIRK:

Took exception to the committee allowing the presentation of this paper.

Considered the paper was brought forward for propaganda purposes.

Department had carried out experiments with a number of types of vaccines made in many ways without any success either prophylactically or curatively. Therefore, considered it unnecessary to re-open the question. Suggested that theoretically there was no reason to expect Streptococcal vaccination to be a successful means of treatment, for no exotoxins were formed as with Scarlet Fever Strep. for production of toxoids which were the most successful of the vaccines. Even with Staph. from which toxoids were prepared, vaccination had not been successful when tried experimentally.

Pointed out that organisms in the udder were still outside the body wall proper and, therefore, not easily accessible for vaccines.

Drew attention to the fact that cows still get severe mastitis in a second or more quarters following infection in a first.

Also stated that workers in laboratories overseas in 1938-39 were not interested in vaccination prophylactically or curatively in any of the places visited by the speaker.

DR. FILMER said that he had listened with interest to the paper. Mr. Marples had pointed out the contradictory results obtained by different workers and had advanced as reasons for this the difference in dose, strain and method of preparation of various vaccines used. It should be remembered, however, that during the period when vaccination for

mastitis was being investigated vaccines had been developed for other diseases about which there was no difference of opinion, many of them giving excellent results. In almost all of these diseases, too, the difficulties in regard to dose, strain and methods of preparation, etc., had had to be overcome. It, therefore, seemed likely that there must be some other reason for the varying results obtained in vaccines for mastitis. He suggested that one explanation might be in the difficulty in diagnosing the disease. Unfortunately, there is no single diagnostic method which is completely satisfactory and no two methods give identical results. It is apparent, therefore, that the results obtained by any worker would depend to some extent on the diagnostic method employed by him.

He agreed with Mr. James and Dr. Hopkirk that vaccines were not likely to be of value in mastitis. The diseases in which vaccines were successful, generally belonged to that class of disease in which an immunity followed recovery from a natural attack; in fact, the very origin of vaccination was due to the observation that dairy-maids who had recovered from attacks of cow-pox were immune to small-pox. Vaccines sought to do what an attack of disease did without subjecting the animal to the risk involved in the natural disease. As no immunity followed an attack of mastitis, it was unlikely, though admittedly not impossible, that vaccination would be successful in controlling the disease.

He expressed disappointment at the fact that the paper writer had submitted no data to indicate that the vaccine referred to in the paper was successful in controlling mastitis. The fact that large quantities of the vaccine were being used should not be accepted as evidence that it was proving effective in controlling the disease as many valueless proprietary remedies enjoyed huge sales. He instanced the example of phosphatic licks for sheep in Australia, which, on the recommendation of those who should have known better (including himself), were used in enormous amounts for a number of years. Recent work, however, had shown that in no part of Australia where experiments were conducted, did such licks have any beneficial effect on either animal health or production.

Summing up, he suggested that the verdict in regard to vaccine for mastitis would be no better than not proven.

DR. H. E. ANNETT:

Dr. Hopkirk's outburst was quite uncalled for and since the paper had been accepted it was no business of his. I considered it an act of great discourtesy on Dr. Hopkirk's part to make such remarks and in my long experience I have never heard anything said concerning a paper read to a Scientific Society which was so lacking in taste. Like many others I had an open mind on the subject matter of the address. Mr. Marples, however, was a well qualified man and it was fortunate that his form had seen fit to employ a man of his calibre. The paper provided an excellent summary of work on mastitis vaccines and it had been prepared and delivered in a manner which might well be emulated by many other scientific workers. It certainly contained no propaganda.

MR. MARPLES was surprised at the muddled thinking regarding the mechanism of vaccines. Certainly a cow could get infection in one quarter, then in a second, then a third and even a fourth, and no natural immunity resulted from one attack. Neither could the cow cure herself. It would be surprising if this were so. But to infer from this that vaccines were useless, would be quite incorrect. The natural infection was purely local and cut off from access to the blood stream. It was not known with certainty which organ of the system took

part in the formation of anti-bodies, but it was known that it was something in intimate connection with the blood stream. By injecting a vaccine, made from the bacteria in the udder, in such a position that it could reach the blood, anti-bodies would be formed and would pass with the blood fluids, into the udder. Mastitis differed from most local infections, in that there was this heavy one-way traffic from the blood stream to the udder.

The criticism of lack of data was quite justified. The data available had been taken from only a small number of herds, and was quite inadequate for scientific proof.

THE CHAIRMAN (PROFESSOR C. P. McMEEKAN) in winding up the discussion, replied to Dr. Hopkirk's contention that the paper should not have been accepted by the Executive , by pointing out that the primary object of the Society was the free discussion of all aspects of animal production. In view of the contentious nature of the subject of the paper, the Executive expected criticism but felt that this was no justification for refusing the opportunity to a member to introduce a subject of such great importance as mastitis, however great the doubts as to the efficiency of vaccines. Mr. Marples was a scientist of repute. The Executive considered that it had no right to refuse his offer of the paper, provided that no suggestion of advertising value was introduced and that the author adhered to the accepted tenets of presentation and matter in scientific papers. However one might disagree with his interpretations and his evidence, members must agree that Mr. Marples had honoured his obligations in these respects.

Continue on page 61.

No page 60.