

weight range show some increase in kidney function related to postnatal age. However, judging from the rate of development of kidney function in larger infants^{3,5} and in rabbits,⁶ the magnitude of the increase observed in our older infants may be less than would be expected in larger infants. In addition, the C_{IN}/C_{PAH} ratios did not change in our infants with increasing clearances. These latter findings suggest that development of kidney function in infants weighing up to 2500 g may be influenced by a gestational factor not operating in larger infants. A more complete de-

scription of these relationships requires direct comparison of these results with similar data on full term infants. Such observations are planned.

Conclusions. Mean values for inulin and PAH clearances and PAH tubular maxima corrected for surface area in premature infants ranged from 17 to 58% of normal adult values. Greater postnatal age appears to increase the rate of development of kidney function in premature infants weighing between 2100 and 2500 g although to a less extent than might be expected in full term infants.

⁵ Gordon, H. H., Harrison, H. E., and McNamara, H., *J. Clin. Invest.*, 1942, **21**, 499.

⁶ Williamson, R. C., and Hiatt, E. P., *PROC. SOC. EXP. BIOL. AND MED.*, 1947, **66**, 554.

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Strains of the Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico.

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(Introduced by R. E. Shope.)

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The present series of papers deals with studies on strains of the virus of foot-and-mouth disease from outbreaks in Mexico. The work was undertaken at the request of the Bureau of Animal Industry of the United States Department of Agriculture on behalf of a joint Mexican-American Commission to whom all the results recorded here have been reported.*

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During the experiments recourse has been made to many new methods that have been developed at this institute, to which some preliminary reference should perhaps be made. The necessity of having a quantitative appreciation of a biological problem is becoming increasingly apparent especially in approaching problems of immunity. No great reliance can be placed on the results of "field" vaccination experiments, for example, in estimating the real value of an immunizing agent as it is not possible to analyse satisfactorily the many factors involved. This can be done only under controlled experimental conditions. Again before satisfactory interpretations can be made of the results of experiments carried out under controlled conditions it is necessary to have reliable "standard methods."

The main program of the scientific staff of this Institute for the last eight years has

been directed with this object in view. As a result it is now possible to measure the potency of virus suspensions of bovine origin such as are used for vaccine production by a method of titration involving the simultaneous inoculation of a number of serial dilutions of virus in the bovine tongue.¹ Sufficient observations are made for the calculation of the 50% end-point. This method guarantees the greatest accuracy commensurate with the use of a very limited number of animals.

In serum neutralization tests correlated with electrophoretic studies and the development of methods for concentrating antibodies it has been shown that by application of the above titration method it is possible to estimate with a greater degree of accuracy than was formerly attainable, the neutralizing and protective properties of bovine antisera and to detect low levels of antibody.² For quantitative estimates of this sort it is obvious that there must be basic information on the relative susceptibility of the test animals employed. In the case of foot-and-mouth disease where cattle are the experimental animals of choice for the study of virus of bovine origin, this susceptibility must vary within wide limits according to the source. There is on the one hand the stock of a country in which the disease is not endemic and in which variations in susceptibility may be attributable to other factors such as age, condition and breed and on the other hand the stock of a country in which the disease is endemic. In this case the "clean history" of the experimental animals is an extremely doubtful factor. This difficulty has been overcome in some countries *e.g.* Holland, by the importation of test animals from a country which is free from the disease.

It should be emphasized that in all the investigations at Pirbright including those under discussion, cattle with a "clean history" and

a relatively uniform level of susceptibility have been employed. While this is indispensable in estimates of virus potency and antibody levels it is equally important in the true appreciation of the limitations of tests for the non-infectivity of vaccines and in estimating the immunizing value or potency of vaccines.

Reference has been made by one of us (I.A.G.) at divers meetings to the results obtained at this Institute in developing standard methods. These included meetings held under the auspices of the Office International des Epizooties in Paris in May, 1947 and in Berne in September 1947,³ the 4th International Congress of Microbiology in Copenhagen in July, 1947 (see Proceedings) and a meeting organized by F.A.O. held at Pirbright in September, 1947. In addition to the methods mentioned above extensive observations carried out on several thousand cattle under controlled experimental conditions have shown that it is possible to estimate the relative potency of different vaccines in contact exposure tests by administering graded doses of vaccine. Results having statistical significance can be obtained, based on the calculation of 50% end-points provided that groups of an adequate number of vaccinated and untreated control cattle are employed. In such circumstances it has been demonstrated that different strains of virus have different immunogenic properties against a strictly homologous virus infection (*i.e.* infection with the same strain) with the implied necessity of selection of suitable strains for vaccination. At the meeting in Copenhagen and again at the 4th International Congresses of Tropical Medicine and Malaria held in Washington in May 1948 preliminary reports were made by one of us (I.A.G.) of some of the experiments, the results of which are embodied in this series of papers. One of the points connected with the immunity problem which has received particular attention has been the difference in antigenic behavior of strains of virus within a main immunological type group.

¹ Henderson, W. M., Thesis, University of Edinburgh, (in press as an Agricultural Research Council Report), 1945.

² Brooksby, J. B., Thesis, University of London, (in press as an Agricultural Research Council Report), 1946.

³ Galloway, Ian A., *Bull. Off. Internat. Epizoo.*, 1947, **27**, 520.

Since these matters were reported another line of approach has been introduced for the detailed study of strains of virus, the application of the complement fixation test. Reference has already been made by one of us⁴ to the development at this Institute of the technic on a quantitative basis.

It is encouraging that already this necessity of developing standard methods on which so much insistence has been placed by the authors is now being widely recognized and their precepts are coming to be adopted more generally.

Paper II⁵ deals with the identification of 13 samples of virus material from Mexico and the accumulated evidence justifies the conclusion that only the virus of foot-and-mouth disease was present in any of the virus samples examined and all the methods employed for the determination of the immunological type involved indicated that all the strains could be placed in the Vallée A group.

There should be some basic information on the pathogenicity and invasiveness of the virus strains concerned before immunity studies are pursued and unless this is forthcoming it is impossible to interpret the results of vaccination experiments correctly. Paper III⁶ summarizes the observations on these characteristics of the Mexican strains of virus. Evidence is produced to illustrate the low invasiveness of these strains. The question has been discussed at some length there as it is of great interest not only because of its epizootological significance but also in the interpretation of the results of vaccination experiments. Further points merit elaboration here. It is often exceedingly difficult if not impossible to secure any information of real value on the origin of a particular outbreak or epizootic of foot-and-mouth disease

and much of the evidence advanced does not proceed beyond the realms of speculation. In this respect the present epizootic in Mexico is no exception.

It is not known how strains of low invasiveness arise. We do not even know whether low invasiveness as determined by tests on a particular breed of cattle of fairly uniform susceptibility under a given set of conditions would be equally demonstrable if observations were made on another breed of different age and conditions in a different climate.

It is known that some species adaptation of the virus appears to occur, for strains of virus of porcine origin sometimes show poor infectivity for cattle. One of us (J.B.B.) has studied such a strain recently and while it spread readily amongst swine it did not do so from swine to cattle. The supposition that the Mexican strains of virus may have originated from swine would appear to be discounted since as has been reported in Paper II,⁵ strain MP (23.12.46) showed no special affinity for swine. On the other hand in countries in Africa, South America and Asia where the disease is endemic and where also susceptible wild animals of different species may roam as in Mexico (*e.g.* deer, wild pigs, goats, llamas, tapirs, porcupines etc. as well as small susceptible rodents of different kinds) it does not seem to be a remote possibility that the disease may spread among these and then return to infect cattle in a modified form due to passage through and perhaps some adaptation to species other than cattle. Where such conditions prevail *i.e.* a virus of low invasiveness is spreading and leaving in its wake animals with a varying degree of immunity; are they not conducive to the development of "variants" and also to the persistence and smouldering of the disease perhaps in a mild atypical form which at certain periods might not be readily recognizable as foot-and-mouth disease? If a virus spreading in this way reaches say "closed" dairy herds of a more susceptible breed of cattle *e.g.* cattle of European origin, this might provide the circumstances and necessary medium for an enhancement of the invasiveness of the strain and a "flare up" which first attracts

⁴ Brooksby, J. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 254.

⁵ Brooksby, J. B., Henderson, W. M., and Galloway, Ian A., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 64.

⁶ Henderson, W. M., Galloway, Ian A., and Brooksby, J. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 66.

attention to the existence of the disease.

Turning to the problem of the interpretation of experiments on vaccination it is apparent from what has been outlined elsewhere^{6,7} that strains of low invasiveness create added difficulties. When in contact exposure tests a considerable number of untreated susceptible control cattle fails to develop visible lesions but on the other hand becomes immunized as a result of infection it is exceedingly difficult to know to what extent the resistance exhibited by the non-reactors in the vaccinated groups can be attributed to the vaccine and to what extent to the low invasiveness of the strain of virus, unless the number of animals in each group is increased beyond the limits of practicability. How much more important this problem becomes in countries where it is difficult to procure cattle of uniform susceptibility! It is known that in countries in which the disease is endemic and where it is not the practice to select animals according to age, breed and condition, it is not uncommon to find quite a number of cattle which will not react even to intradermal inoculation of the tongue with massive doses of strains of virus of high invasiveness. It would therefore not be surprising if the number of non-reactors to such inoculations was not materially increased when strains of low invasiveness such as the Mexican strains are under investigation. The significance of the virus-host relationship is further illustrated by the facts which emerge from the observations on the titration of two of the Mexican strains of virus, MP and M.1 (in suspensions prepared from the vesicular tongue lesions or in the circulating blood) referred to in Paper III.⁶

It is obvious that unless there is awareness of the influence of the factors discussed above there can be no satisfactory approach to a discussion of what are sometimes glibly referred to as "international standards".

When in vaccination experiments there are indications that the invasiveness of the strain of virus under investigation is low and the

susceptibility of the cattle is nevertheless relatively and uniformly high, it is possible as has been recorded in Paper VI⁷ to use another method of estimating the potency of a vaccine. This is dependent on observations on the occurrence or non-occurrence of secondary lesions on the lips or feet or elsewhere following upon the inoculation of virus intradermally into the tongues of groups of vaccinated and control cattle. As in contact exposure tests little reliance can be placed on observations on too small groups of cattle for as will be seen from the results secondary lesions sometimes do not develop in the untreated control cattle.

Further work is being done to find out the correlation between the results in potency tests of vaccination with graded doses of vaccine followed by exposure to infection by contact and similar potency tests in which the vaccination with graded doses of vaccine is followed by exposure to infection by inoculation of virus intradermally into the tongue or by other routes.

Particular attention must be drawn to the results of experiments which are recorded in Papers IV,⁸ V⁹ and VI⁷ illustrating the antigenic behavior of the Mexican strains of virus MP and M.1 in relation to 119 the Pirbright stock strain of virus of the main Vallée A immunological type.

It is perhaps as well to deal first with the observations which were made in vaccination experiments with virus strains MP (Mexican) and 119 (Pirbright). When it was found that the first samples of virus sent from Mexico including strain MP were of the Vallée A immunological type, an experiment was set up to determine whether a vaccine prepared from strain 119 (Vallée A) would give as good protection to cattle exposed to infection with the Mexican strain MP as to cattle exposed to infection with strain 119. From observations as yet unpublished carried

⁷ Henderson, W. M., Galloway, Ian A., and Brooksby, J. B., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, **69**, 77.

⁸ Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, **69**, 70.

⁹ Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., *PROC. SOC. EXP. BIOL. AND MED.*, 1948, **69**, 74.

out under strictly controlled experimental conditions on 320 vaccinated cattle and 116 untreated control cattle in a series of experiments, it has been proved that strain 119 is of high immunogenicity against strictly homologous virus infection (50 per cent end-point protection dose = 8 cc). As will be seen from the results of the recorded experiments in Paper VI,⁷ a vaccine prepared from strain 119 did not, when administered in equivalent dose, give the same measure of protection against infection with strain MP as it did against infection with strain 119.

When this result was obtained an examination of the two strains was made in cross serum neutralization tests in cattle, Paper V,⁹ and it was found that bovine anti-sera had a greater neutralizing effect on strain homologous than on strain heterologous virus. In complement fixation tests also, Paper IV⁸ differences were apparent in the antigenic behaviour of strain MP and strain 119 when anti-sera from guinea pigs infected with the respective strains were employed.

Cross serum neutralization and complement fixation tests were then carried out with strains MP and M.1 and differences were exhibited also in the antigenic behaviour of these strains both in relation to strain 119 and to one another. Since strains MP and M.1 appeared to differ from one another in their behaviour in complement fixation and serum neutralization tests an investigation of their antigenic behaviour in cross immunity or vaccination experiments was made. As will be seen in Paper VI⁷ the results of these preliminary tests were not conclusive although their trend suggested some difference between strain MP and M.1. It is conceivable that in similar experiments in which groups of cattle are tested by the same method after injection with smaller graded doses of vaccine it may be possible to bring out greater differences in the antigenic behaviour of these strains. It is obvious that further work is necessary to clarify the position and that all due caution must be exercised in drawing conclusions. However, even at this stage, there is justification for the statement that the results of the examination of the antigenic behaviour of the Mexican strains of virus

has strengthened the evidence for the occurrence of "variants" within the main Vallée A immunological type group. The use of the term "variant" is one of convenience only and in this instance is for the purpose of describing some observed difference in the antigenic behaviour of strains of virus which are so similar in their behaviour in general immunological typing tests that they can clearly be classified only in one particular type group. This class of "variant" is distinct from others apparently more complex which has been described by Traub and his co-workers^{10,11} as the result of complement fixation tests. These latter variants show some partial relation to other immunological type groups. There appears to be correlation in some instances at least between the differences brought out by the three methods of study of antigenic behaviour but a considerable amount of work is now necessary to examine as many strains of virus as possible to see whether this is universally the case. Particular attention is being paid to the strains of virus regularly employed for vaccine production in the different European centers. It would be tempting to assume that because agreement in the results of complement fixation, serum neutralization and cross-immunity tests has been observed in the experiments recorded here it would be possible to forecast the results of vaccination experiments with a strain of virus from the results of the other two simpler tests. It is premature to assume that this would be so. It would be reasonable to suppose that some of these antigenic differences may be quantitative and that the limited range of antigenicity of certain strains may be compensated for by increasing the amount of antigen administered in vaccination or again some of the difficulties may be overcome by the selection of strains of good immunizing power and of a wide range of antigenicity. The real significance of the occurrence of these "variants" within an immunological type group has yet to be assessed. What is strongly suggested

¹⁰ Traub, E., and Rodriguez, R., *Zbl. Bakt. Abt. 1, Orig.*, 1944, **151**, 380.

¹¹ Traub, E., and Möhlmann, H., *Berl. und Münch. tierärztl. Wschr.*, 1946, **1**, 1.

is that these observations cannot be ignored in immunization procedures since Traub and Möhlmann¹¹ while working in Europe recovered some similar Vallée A "variants" from some cattle which developed the disease although they had been vaccinated in the field with a stock bivalent vaccine in which two strains of virus, one of the Vallée O type, and the other of the Vallée A type were incorporated. The cattle in these "vaccine breaks" had been vaccinated with a Schmidt-Waldmann aluminum hydroxide vaccine.

Their method of approach to the problem was different from that of the present authors in that they came across these "variants" while carrying out complement fixation tests. They did however add a rider to the effect that although the late war had prevented them from being in a position to place their observations on record, they had found that the Vallée A strain of virus used for vaccine production was not so immunogenic against the other "variants" as it was against itself and the same held true for the behaviour of the other "variants."

It is apparent, and this was stressed at the 4th International Congress of Microbiology in Copenhagen, that the position with regard to strains of virus of foot-and-mouth disease is similar to that which appears to exist with strains of influenza virus *i.e.* "variants" occur within a main immunological type group. Where workers on foot-and-mouth disease have an advantage over those engaged on the influenza problem is that although for obvious reasons progress is bound to be slower, the strains of virus can be studied in the host from which they were recovered and cross immunity vaccination experiments involving exposure to infection with a selected strain of virus can be carried out on cattle in which the disease occurs under natural conditions.

In the present series of experiments the Mexican strains MP and M.1 and the stock strain 119 were all strains of bovine origin. The serum neutralization tests were made with bovine antisera and these and the cross immunity tests were carried out in cattle. Until some means is devised of overcoming the dif-

ficulties encountered in using bovine antisera in complement fixation tests, virus strains have to be adapted to guinea-pigs for the preparation of antisera for this purpose. It would be preferable to avoid this procedure. As a result of the observations recorded above it was recommended to the Bureau of Animal Industry of the U. S. Department of Agriculture for the benefit of the joint Mexican-American Commission that until further information became available on the range of immunogenicity and specificity of other Vallée A type strains of virus it would be preferable to use Mexican strains of virus for vaccinating in Mexico (Results of experiments in which cattle injected with vaccines prepared from Mexican virus strains were subsequently exposed to infection with the same strains are recorded in Paper VI⁷). When again from the results of experiments some antigenic differences appeared to exist between the Mexican strains MP and M.1 it was recommended that until further information was obtained both these strains should be incorporated in vaccines for use in Mexico, in fact a bivalent vaccine incorporating MP and M.1 should be employed. The position is however in a constant state of flux and it must be reviewed from time to time.

This is neither the time nor the place to discuss fully the observations recorded in this series of six papers in relation to the control of foot-and-mouth disease. The procedure must be governed by the conditions obtaining. Attention might however be drawn to the more obvious implications. Every effort should be made to prevent foot-and-mouth disease becoming endemic in a country. This can be accomplished in certain exceptional circumstances by a "stamping-out" policy alone involving the early and rapid slaughter and disposal of carcasses by burial or burning and the application of a "cordon sanitaire" including police and quarantine measures.

If vaccination has to be introduced, owing to the failure or inapplicability of a "stamping out" policy, either alone or in combination with slaughter in zonal control measures or on a more extended basis then rapidity is

again the key to the situation and every effort should be made to induce as high a grade of immunity in as many susceptible animals as possible in as short a time as possible.

Whatever the class of vaccine to be employed, there must be information obtained on the strain or strains of virus responsible for the outbreaks.

Furthermore a phase has now been reached in research on the disease which points to the necessity of "standard methods" such as those referred to above so that as highly effective vaccines as possible can be produced. This involves the selection of strains of good immunogenicity and of as wide a range of group antigenicity as possible. The last word on vaccination against foot-and-mouth disease has not yet been said. If when the disease is already, or should owing to untoward circumstances, become endemic new aspects of the control problem arise but this theme cannot be developed here. However something should be said about one or two points of interest. Under epizootic or endemic conditions is it not likely that some reported cases of "reinfection" in herds which were known to have passed through the disease only a relatively short time before may be due to infection, with the same strain of virus, of cattle which escaped the disease before, or at least acquired only a low grade of immunity due to the low invasiveness of the strain? Another possibility is that a strain "variant" may have arisen. It is often assumed without further investigation that such cases of so-called "reinfection" are due probably to

another immunological type. In at least one specific case of reported "reinfection" during the Mexican epizootic such a belief was not substantiated by the examination of the virus sample collected. This aspect requires further study.

Examination of samples of virus from outbreaks during the course of an epizootic has of necessity in the past been very fragmentary. The observations recorded here suggest strongly the advisability of a more detailed and continued examination of as many virus samples as possible. This would contribute to a better understanding of the epizootiological picture and the sequence of events. Indeed it would be essential if effective control and possibly ultimate elimination of the disease was the aim in view.

Nothing has been said about the possibility of the occurrence of other vesicular diseases such as vesicular stomatitis or vesicular exanthema at the same time in areas where foot-and-mouth disease is spreading but that is another question.

The problem of the attempted control of foot-and-mouth disease by procedures involving vaccination is obviously more complex in some cases than is generally appreciated but not so complex that continued scientific endeavor may not introduce considerable improvements in many directions. It should be apparent also that in applying methods of control involving vaccination, continual guidance and indeed direction of the program must come from the laboratory.