

## THE CONGLUTINATION PHENOMENON

### III. THE CONGLUTINATING COMPLEMENT ABSORPTION TEST IN EXPERIMENTAL GLANDERS

BY N. H. HOLE AND R. R. A. COOMBS\*

*From the Veterinary Laboratory, Ministry of Agriculture and Fisheries, Weybridge, and  
The Department of Pathology, University of Cambridge*

(With 6 Figures in the Text)

#### CONTENTS

	PAGE		PAGE
I. INTRODUCTION . . . . .	497	(c) Course of the experiment . . . . .	498
II. EXPERIMENTAL STUDY . . . . .	497	(d) Results . . . . .	499
(a) Materials . . . . .	497	III. DISCUSSION . . . . .	499
(b) Methods . . . . .	498	IV. SUMMARY . . . . .	503
		REFERENCES . . . . .	503

#### I. INTRODUCTION

Glanders was the first disease to which we have applied the conglutinating complement absorption test. The opportunity for doing this was afforded during the war when the serological diagnosis of glanders in horses became sufficiently important to justify a programme of research on this subject.

The purpose of this paper is to record these investigations, which clearly show the value of this reaction for demonstrating the immune blood response in this disease.

The conglutination reaction has long been associated with the serodiagnostic procedures which may be used in this condition and, for earlier publications on this subject, reference should be made to the paper by Streng in 1929. The test was usually considered a useful supplement to the haemolytic complement fixation test, although it was often stated that the haemolytic test gave a clearer differentiation between a positive and a negative result. The reason for this statement may perhaps be explained if the technique used in those early days is borne in mind. The reaction found its greatest application in the examination of the sera of asses and mules as these sera were often too anticomplementary for guinea-pig complement to be examined by the haemolytic complement fixation test. Some workers were of the opinion that the conglutination reaction was of special value in the diagnosis of chronic glanders, although this was denied by others.

In this series of experiments ponies were treated orally with a virulent culture of *P. mallei*. The rise and fall of complement fixing antibodies, as demon-

strated by the conglutinating complement absorption and haemolytic complement fixation tests, were followed over a period of 10 months. Agglutination tests were also performed on the serum samples.

Some additional data are included on the effect of allergic tests on the blood antibody level.

#### II. EXPERIMENTAL STUDY

##### (a) Materials

Dartmoor ponies were used in these investigations, which were begun on 24. ix. 43, when ponies 3, 4, 5, 7 and 8 were treated *per os* with virulent cultures of *P. mallei*.

The organism was strain K 199, which had been passaged through donkeys several times; 0.1 c.c. of a broth culture of this strain, given orally, killed the donkeys with generalized glanders within 20 days. The fact that donkeys, unlike horses, generally succumb to acute glanders after experimental infection is well known.

Pony 6 and later ponies 11, 12 and 13 acted as control animals. Ponies 1 and 2, which had been treated *per os* with *P. mallei* on 4. v. 42, were also included in the experiments.

Previous history. Allergic tests (I.D.P. = intradermo-palpebral test; s.c. = subcutaneous test) with mallein had been performed on some of these ponies as follows: 23. iv. 42 I.D.P. ponies 1, 2, 3 and 4; 18. v. 42 I.D.P. and s.c. ponies 1, 2, 3 and 4; 28. v. 42 I.D.P. and s.c. ponies 3 and 4; 2. vi. 42, 13. x. 42 and 8. xii. 42 I.D.P. and s.c. ponies 1 and 2; 20. iii. 43 I.D.P. and s.c. ponies 1, 2 and 4; 9. ix. 43 s.c. four different malleins ponies 2 and 3.

\* John Lucas Walker Student. This work was performed when this author held a grant under the Agricultural Research Council.

(b) Methods

The serum samples were tested by means of the conglutinating complement absorption and haemolytic complement fixation tests for the presence of complement fixing antibodies. The techniques used were those described in the previous paper (p. 490).

The pre-inoculation titre for the agglutination test varied between 80 and 640. The presence and wide variation in titre of normal agglutinins in horses for *P. mallei* are well-established facts.

On 24. ix. 43.0.5 c.c. of a broth culture of *P. mallei* was placed on the tongue of ponies 3, 4, 5, 7 and 8.

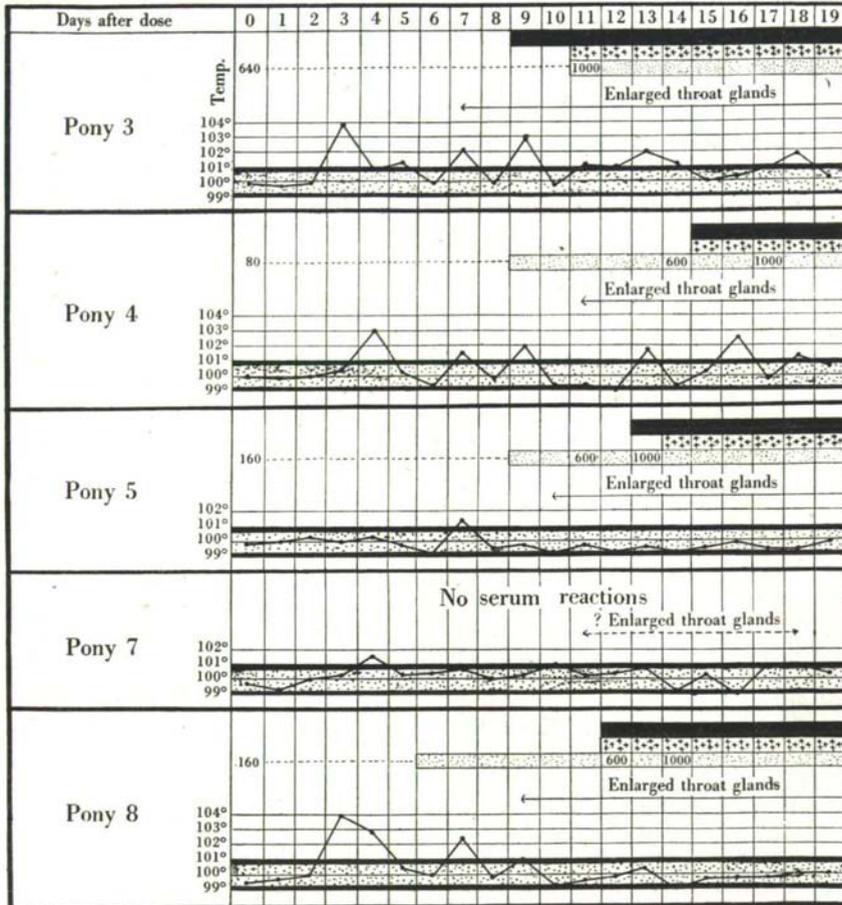


Fig. 1. Initial serological and thermometrical reactions to the oral administration of *P. mallei*. ■ conglutinating complement absorption; ▨ haemolytic complement fixation; ▩ agglutination above pre-inoculation titre; — normal temperature range.

The antigen was a Seitz E.K. filtrate of an autoclaved 6 weeks synthetic broth culture of the organism. The lowest serum dilution tested was 1:10. Guinea-pig complement was used in the haemolytic tests and horse complement in the conglutination tests. Agglutination reactions were also performed using a suspension of *P. mallei* as antigen.

(c) Course of the experiment

The animals were bled previous to the infective treatment and the sera were shown to give no reactions by the complement absorption tests.

Blood was drawn daily for 26 days, and thereafter bi-weekly. After allergic tests blood samples were taken daily for 10 days.

The allergic tests performed on the ponies during the course of the experiments, as shown in the histograms, were carried out to observe their effect on the blood picture.

At the end of the experiment the animals were slaughtered. Post-mortem and histological examination revealed no lesions of active disease, and in ponies 1, 2 and 5 nothing abnormal was seen that could be associated with glanders. Pony 8 showed

small typical chronic glanderous lesions in the lung, and in ponies 3 and 7 very suspicious small encapsulated lesions were present in the liver parenchyma. Pony 4 had a typical small single lesion of chronic glanders in the submaxillary lymph gland that was only found on microscopical examination. The small size of this lesion and the fact that it was missed macroscopically suggest that other ponies may have had similar small lesions, which were overlooked.

antibody level was a point of special interest because of the possibility of using this observation as an aid to diagnosis. Fig. 6 is a histogram demonstrating the rise in the antibody above the existing level after the injection of mallein by the intradermo-palpebral and subcutaneous routes.

Fig. 6 demonstrates the rise in the existing serum antibody level produced by mallein allergic tests. Histograms for ponies 3, 4, 5 and 8 are taken from Figs. 2-5.

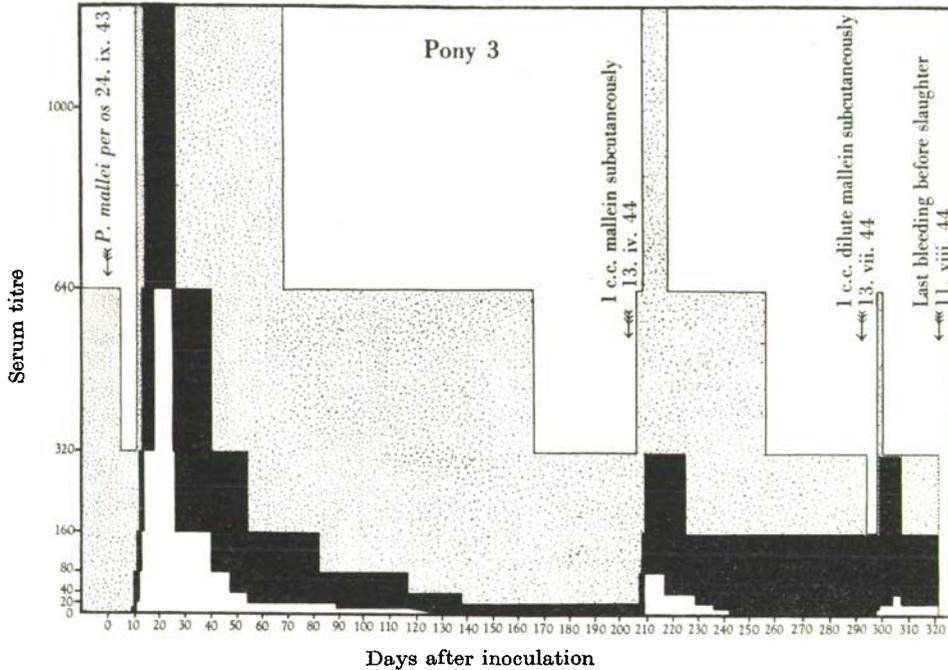


Fig. 2. Pony 3. Serological response to oral administration of *P. mallei*. [stippled] agglutination; [solid black] complement absorption; [white] haemolytic complement fixation.

Post-mortem examination therefore revealed definite evidence of chronic glanders in ponies 4 and 8, very suspicious lesions in ponies 3 and 7 and no evidence of glanders in ponies 1, 2 and 5.

(d) Results

The results of the serological examinations are summarized in the following histograms. Fig. 1 records the first day after inoculation, on which definite positive reactions were obtained with the different serological tests. The demonstration of antibodies over the period of the whole experiment is recorded in Figs. 2-5. No antibodies were detected in pony 7 in response to the treatment with *P. mallei*, but the injection of mallein 203 and 292 days later resulted in the temporary appearance of antibodies in high titre.

The effects of the allergic tests on the blood

III. DISCUSSION

Altogether seven ponies were treated orally with *P. mallei* and were the subjects of the serological examinations. At post-mortem no lesions of active glanders were observed and old encapsulated lesions found in only four of the animals. However, there seems to be little doubt that all the animals were infected at some time during the course of the experiment, because they all developed demonstrable serum antibodies with one exception, and this animal subsequently gave a positive allergic reaction and had a suspicious lesion at post-mortem.

The comparative value of the different serological methods used is the main subject for discussion. The agglutination test on account of the variable normal agglutinin titre cannot be considered a method of choice. The examinations carried out clearly indicate the suitability of the complement

*The conglutination phenomenon*

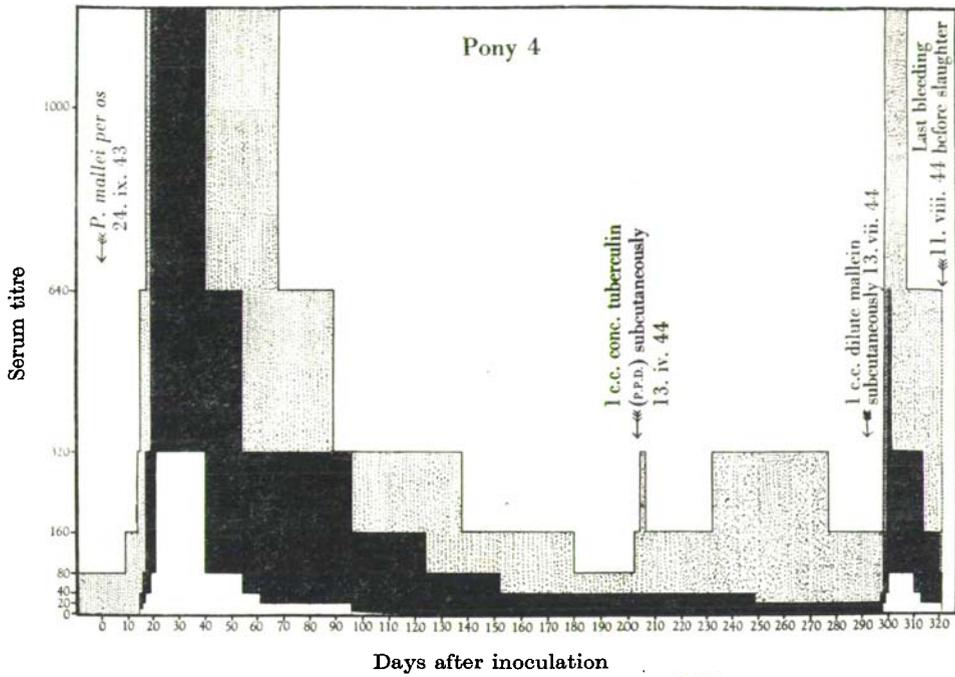


Fig. 3. Pony 4. Serological response to oral administration of *P. mallei*. [stippled] agglutination; [black] conglutinating complement absorption; [white] haemolytic complement fixation.

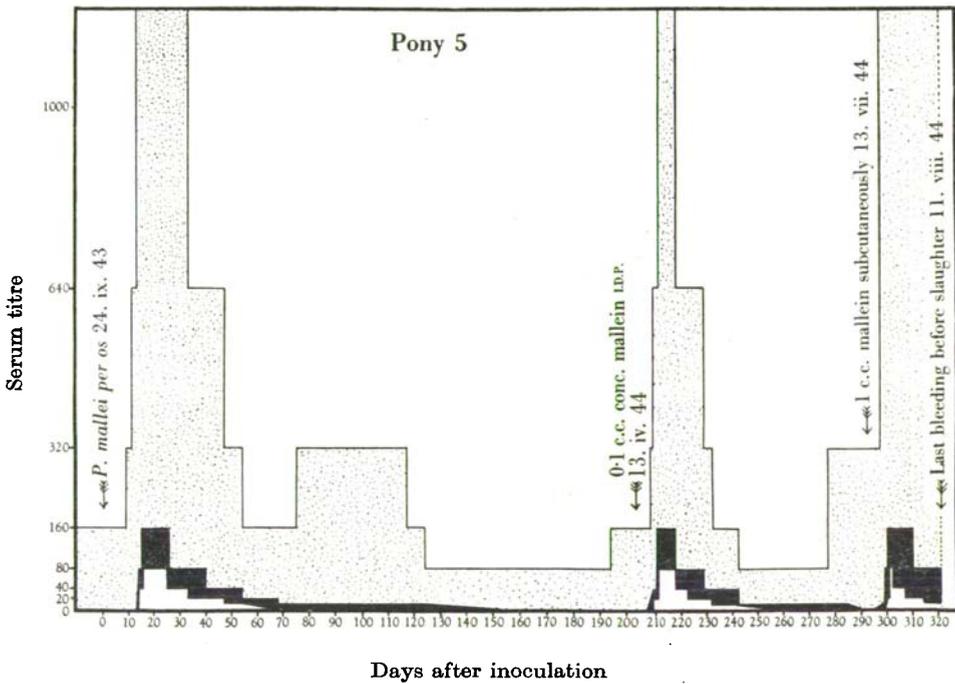


Fig. 4. Pony 5. Serological response to oral administration of *P. mallei*. [stippled] agglutination; [black] conglutinating complement absorption; [white] haemolytic complement fixation.

absorption test for the active and regressive stages of the disease. In comparing the results, the advantages claimed for this reaction may all be dependent on its detecting antibodies to a higher titre than that obtained by the haemolytic complement fixation test. This greater sensitivity of the conglutination reaction must be due to the nature of the complement used, for were it not for this, it would be expected that the higher titre would be demonstrated by the haemolytic reaction, as in this case the complement titration is based on initial haemolysis, while in the conglutination reaction it is based on complete conglutination.

glutinating complement absorption test shows to advantage over haemolytic complement fixation in the detection of the initial antibody response; the antibodies were never detected later by the former reaction and in two cases were observed earlier—2 days in the case of pony 3 and 1 day in the case of pony 5. A greater number of tests is desirable before a definite statement on this point can be made. The high titre, the early response, and the absence of reaction in normal animals, place the conglutinating complement absorption test, in our opinion, as the best serological means of diagnosing recent infection. According to Hutyra, Marek & Manninger (1938)

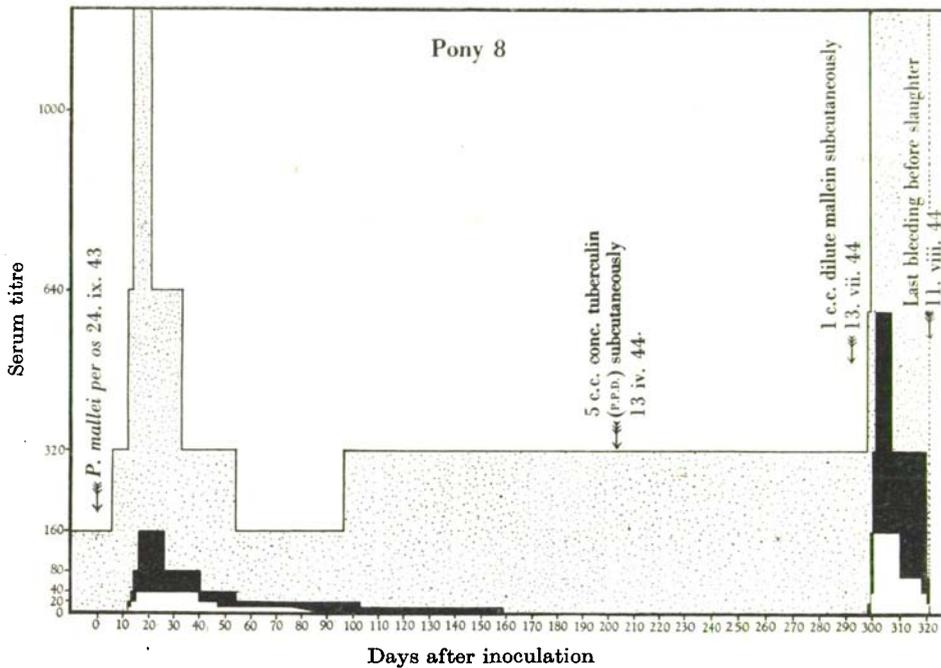


Fig. 5. Pony 8. Serological response to oral administration of *P. mallei*. ■ agglutination; ■ conglutinating complement absorption; □ haemolytic complement fixation.

The initial antibody response to the infection, detected by the respective serological methods, is shown in Fig. 1. Agglutinins are the first antibodies to show a detectable rise above their pre-inoculation level. This might be expected in view of the fact that antibodies of this nature and configuration already exist in the normal serum, and also because agglutinogens are presumably antigens situated at, or near the surface of the bacterium. The value of this rise for the interpretation of isolated diagnostic tests, however, is vitiated by the uncertainty of the pre-inoculation titre. The authors have never observed a positive reaction with the sera of over fifty normal horses, using either of the complement absorption tests. Of these reactions the con-

glutination test is equal to the complement fixation test as regards reliability, but gives positive reactions only in the later stages of the disease. . . . This is not the experience in the experiments here recorded.

At no time over the whole experiment were antibodies detected to a higher titre by the haemolytic test than by the conglutination reaction. Moreover, by the use of this latter procedure antibodies could be demonstrated in the serum for a long time after they could no longer be shown by the haemolytic test. Pony 7 was peculiar, for, although it was reasonably certain from the histo-pathological examination and results of allergic tests that it had been infected with glanders, no serum antibodies

could be detected except for short intervals after the allergic tests. The possibility of 'incomplete' or 'univalent' antibodies (Tyler, 1945) being present, but not demonstrable, is brought to mind.

The third point for discussion is the effect of allergic tests on the blood picture of sensitized animals. Mallein, particularly when given subcutaneously, has long been recognized as antigenic for normal animals and this is one reason why the

A heterologous allergen such as tuberculin, even in large doses, had no effect, but mallein, as was expected, stimulated a marked rise in serum antibodies in sensitized animals, and these results, together with the effect produced in control ponies, are shown in Fig. 6. This matter is a question of considerable interest and deserves further investigation as it would appear that for the surest diagnosis of glanders a combination of both blood and allergic

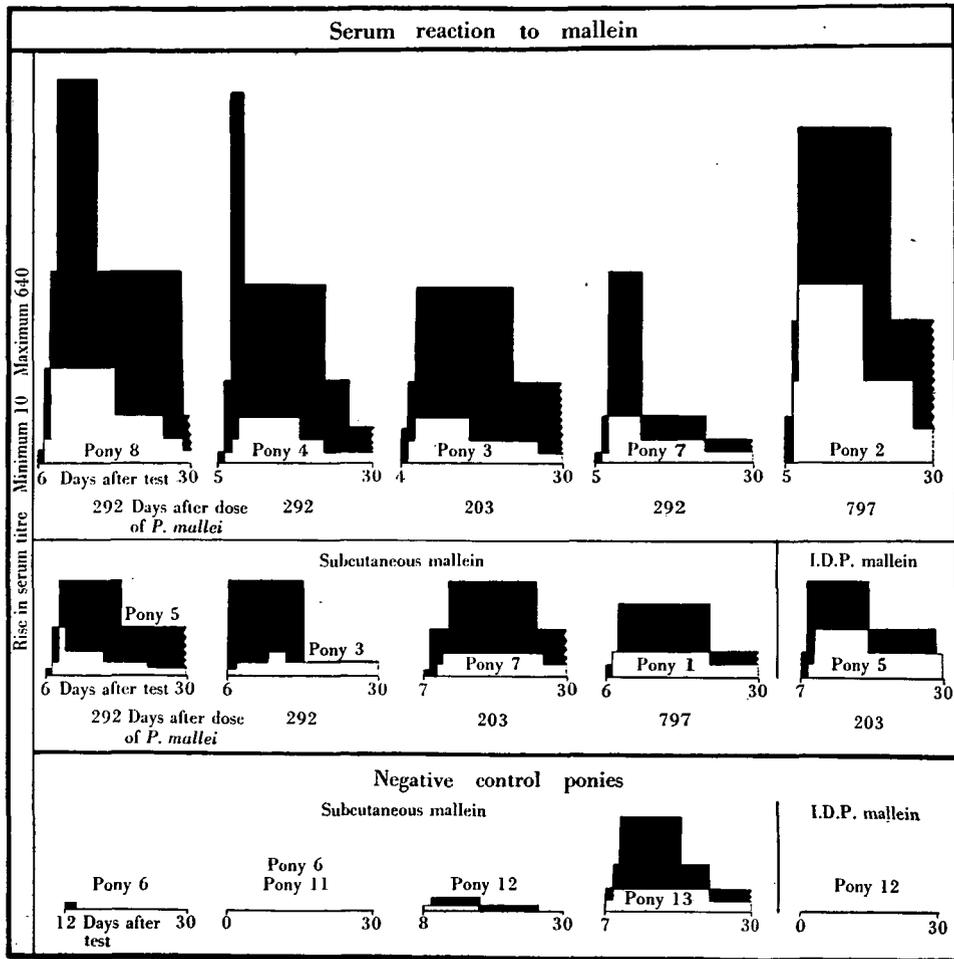


Fig. 6. Serum reaction to mallein. Sensitized ponies after subcutaneous mallein tests. ■ conglutinating complement absorption; □ haemolytic complement fixation.

subcutaneous allergic test is no longer used in many parts of the world. The ophthalmic test is said to have no antigenic effect and the intradermo-palpebral (I.D.P.) test only a very slight action in this respect. Schnürer (quoted by Hutyra, Marek & Manninger, 1938) reports that agglutinins are increased in glanderous but not in normal horses by the ophthalmic mallein test.

tests is required. The results of allergic tests are often doubtful. Our interpretations of the allergic tests shown in Fig. 6 were in the order illustrated, as follows: doubtful, positive, positive, positive, doubtful, positive, doubtful, doubtful, positive, positive.

The blood response to these mallein tests was greater in sensitized animals than in the controls. In the case of the subcutaneous test the antibody

demonstrated by the complement absorption tests showed a considerable rise in titre in the sensitized animals but, although no serum antibodies appeared in two controls, three other control animals showed the appearance of antibodies to a titre of 10, 20 and 160. In the case of the I.D.P. test one sensitized pony, whose antibody level had been below 1 : 10 for over 60 days before the test, developed a serum titre of 160, while eleven control animals (only one, namely pony 12, is shown in Fig. 6) after a similar allergic test showed no appearance of antibodies. This observation, if later confirmed, might afford an extra diagnostic procedure for this disease.

In conclusion it may be stated that, in this limited study, the conglutinating complement absorption test has compared more than favourably with the ordinary haemolytic complement fixation test in the demonstration of serum antibodies in ponies during an experimental glanders infection.

#### IV. SUMMARY

1. Observations on the sera of ponies, taken at frequent intervals for 321 days after oral administration of *P. mallei*, are described.

2. It was found that the conglutinating complement absorption test was more sensitive than the haemolytic complement fixation test as a means of diagnosis. It detected the antibodies earlier in the course of the disease and demonstrated their presence over a longer period of time.

3. The possibility of another practical use of this reaction as an adjunct to the allergic test is considered. Ten days after an intradermo-palpebral test a pony, which had been previously sensitized and whose serum antibody titre at that time was below 10, developed a serum titre of over 160 as demonstrated by the conglutinating complement absorption test. Under similar circumstances 11 unsensitized ponies developed no detectable serum antibodies.

The authors would like to express their thanks to Mr H. P. Hudson of the Department of Pathology, University of Cambridge and to Mr A. Norman of the Veterinary Laboratory, Ministry of Agriculture, Weybridge, for photographing the histograms and tests reproduced in these articles.

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(MS. received for publication 19. v. 47.—Ed.)