had formed an important and extensive study. He stated that these cases often appeared in herds that had been tuberculosis free for some time and in which no contact with tuberculosis cases had occurred. The lesions were found on the legs, shoulder or teats. Work carried out on organisms isolated from these lesions showed that they were definitely acid-fast in character and caused reactions to tuberculin that were not always typical. Marsh, Warren and Morrow (1932) concur with these statements and assert that the subcutaneous lesions are almost confined to milking cattle and are usually to be found on the teats. Acid fast organisms can frequently be demonstrated from these lesions and if injected into small animals can occasionally set up lesions from which acid fast organisms can be recovered. Cultural efforts in their hands were unsuccessful and they express a doubt as to whether they are M. tuberculosis or not. When tested with bovine tuberculin intradermally animals that show these lesions give varying and unstable positive reactions, to the test. No reactions are observed when the subcutaneous or the ophthalmic tests are applied and little if any response is shown when avian tuberculin is injected intradermally. From evidence submitted these subcutaneous lesions do not appear to be able to resolve into internal tuberculosis.

In a report by the Chief, Bureau of Animal Industry, United States of America for 1933 it was stated that further investigation into the skin lesion problem was carried out and two acid fast organisms isolated. When these were injected into test animals similar skin lesions were caused but they healed up after 4 months. During these four months these animals were sensitive to the tuberculin test.

Daines and Austin (1933) isolated 3 types of organisms from so-called 'skin lesions' :-

(a) acid-fast diphtheroid forming dry rough colonies on agar, this most frequently caused nodules when injected into animals.

(b) acid fast diphtheroid forming moist glistening colonies on agar.

(c) a non-acid fast organism showing great pleomorphism.

Some cows injected with these organisms showed skin lesions some of which were typical. Intradermal tests with tuberculin on these cattle gave reactions that varied from negative to suspicious. The authors suggest that organism (a) is an undescribed new species.

Continuing their work these same two authors (1934) isolated 4 groups of organisms from skin nodules of tuberculin reacting cattle.

(1) an occasional bovine M. tuberculosis strain.

(2) a partially or non-acid fast pleomorphic chromogenic bacillus.

(3) a bacillary pleomorphic acid-fast bacillus which was an orange coloured chromogen.

(4)/....

(4) a non-acid fast, non-chromogenic pleomorphic bacillus.

Injections into cattle of organism No. 3. caused apparently typical skin lesions as a result of repeated inoculations of this organism. Two of these cows so inoculated gave definite but transitory Tuberculin reactions and pronounced allergic reactions with a protein filtrate of No. 3. organism. They suggest that No. 3. group of organisms is the cause of skin lesions and that at the same time a transitory sensitiveness to tuberculin is also set up.

Feldman (1934) made a histological study of the so-called 'skin lesions' that occur in the subcutaneous tissues of cattle. This condition appeared to be widely distributed throughout the United States of America. Thirty four specimens of subcutaneous nodules were obtained from cattle that had given positive tuberculin reactions but which on post-mortem examination had revealed no lesions of Tuberculosis. These specimens were irregular nodular masses varying in size from 1 to 4 cms. in diameter, and they were situated in the corium and underlying subcutaneous tissues. More than half of them showed varying degrees of calcification. From 22 out of the 34 specimens acid fast organisms were obtained. In some of these the bacilli were numerous, in others, few. In addition, in the cell debris some non-acid fast organisms were seen. Histologically the lesions bore a considerable resemblance to lesions of tuberculosis. The conclusion drawn was that M. tuberculosis was not the causal organism.

Rautmann (1934) gave a list of sites of tuberculous lesions found on post-mortem examination of 190 positive cases of Tuberculosis - no mention of any skin lesions is made.

Lobel (1934) described a nodular skin condition in buffaloes that was situated mainly in the corium. Acid fast bacteria and granules were found in the fatty contents of the nodule. These nodules may show foci of necrosis or calcification. He was not able to cultivate this organism. Eight of these affected buffaloes were tested with bovine tuberculin by the ophthalmic method and three gave positive reactions. When tested by the subcutaneous method using bovine and avian tuberculin no reactions were obtained. The author concludes that this condition has nothing whatever to do with Tuberculosis or the skin nodules of tuberculin reacting cattle described largely in America. It resembles rather nodular Leprosy of man and has therefore termed the condition 'lepra bulborum.'

That skin lesions caused by M. tuberculosis are not confined to cases in cattle is confirmed by Aramy (1934) who stated that post mortem examination of positive reactors to the tuberculin test in pigeons revealed lesions usually in the joints and in the skin.

Prendergast (1936) stated that as a result of a survey of a very large number of tuberculin reacting cattle bearing skin lesions he failed on post-mortem examination to find internal lesions of Tuberculosis in 95% of these cattle. He suggested that in these instances the intradermal reactions were not altogether typical.

Crawford/....

Crawford (1936) made reference to skin lesion cases and claims that the organisms found in these lesions do cause a sensitivity to tuberculin. He isolated two new acid fast organisms of this type and in animals inoculated with them 'skin lesions' were obtained. When these animals were testedwith Tuberculin reactions were irregularly obtained - with avian tuberculin the reactions were stronger. From one of these strains a 'tuberculin' was prepared and gave some positive reactions in tuberculous cattle and in cattle showing 'skin lesions'. The reactions of these cattle were more marked however, when tested with American Standard tuberculin.

Robertson and Hole (1937) described 12 'skin lesion' tuberculous reactors. On post-mortem examination nothing could be found internally and a biological examination also gave negative results. In 11 cases out of 12 acid fast organisms were isolated from the nodules. On media commonly used for the isolation of M. tuberculosis these organisms would not grow and animal inoculations were all negative. The conculsions come to by these authors were that these organisms caused a sensitization to tuberculin but were not necessarily the primary cause of the lesions. They admit the possibility of these organisms being an atypical M. tuberculosis but incline rather to the view that they were probably of the saphrophytic type of organisms causing a group sensitisation to tuberculin. Brook (1937) refers to a condition in the United States of America and Canada known as 'skin tuberculosis' and states that it received this name because a proportion of these cattle react to the several tuberculin tests although on post-mortem examination the animals are usually found to be free from macroscopic signs of orthodox tuberculosis. He quotes Hagan (1930) who stated that Perard and Ramon (1913) were the first to describe this condition. He gives a good description of the lesions seen and mentions the site of their occurrence. From his investigations he stated that most of these cases seen were in herds that were free from orthodox bovine tuberculosis and that a few cattle only were affected in a given herd at one time. Communications that he had received from field veterinary officers stated that if these lesions were removed surgically the animal lost its sensitivity to tuberculin. In personal communications from leading authorities in Sweden, Finland, Denmark, Germany and Switzerland, they all state that the above mentioned condition is unknown in their respective countries.

STANDARDISATION OF TUBERCULIN - In order to obviate doubtful reactions it has been asserted that Tuberculin for use in animals should be of a definite standard. All tuberculin for human use is standardised according to Frankfort standard which is the old standard of Erlich. In the Union tuberculin is standardised against the Frankfort standard and the strength of tuberculin recommended for field work should be at least slightly stronger but twice standard should be aimed at in order to obtain the best results.

In the second report of the Tuberculin Committee to the Medical Research Council (1928) two recommendations

were/....

were made as the result of further experience in the carrying out of the Tuberculin test, viz. that an increased strength of tuberculin might be employed. It was suggested that the actual dose (.1 c.c.) should not be increased but a more potent form of tuberculin should be utilized. The second recommendation was that in order to obtain consistent results a standardised tuberculin should be used.

Buxton (1928) in an appendix to the above report gives a method of standardisation - the standard he used was his own and apparently he was not using the old Frankfort standard. Glover (1931 and 1932) as a result of having carried out a large number of double intradermal tests on cattle stressed the desirability of utilizing a standardised tuberculin.

de Kock (1932) laid down that in South Africa the test should be undertaken only under special conditions and one of these was 'all tuberculin tests in any scheme adopted should be carried out with properly standardised tuberculin'. This was in an effort to eliminate the doubtful reactor to the test.

Glover (1933) being of the opinion that non-specific reactions to the double intradermal test might be caused by various ingredients contained in the media on which tuberculin was prepared, set out to manufacture tuberculins on synthetic media. These he purified by chemical means. On testing these tuberculins out on tuberculous cattle under Laboratory conditions he found that they were not less potent than a 'standard' tuberculin. In negative animals the reactions produced by the tuberculins produced on synthetic media gave an appreciably smaller reaction than did the tuberculin produced on the usual media.

Buxton and Glover (1933) confirmed these Laboratory results by using the tuberculins prepared by their methods on cattle under field conditions. Dorset (1934) compared the 'old' tuberculin of Koch with tuberculin prepared on a synthetic medium. A few more reactions were given to the synthetic medium tuberculin than to the 'old' tuberculin, but the percentage of 'no lesion' reactors on autopsy was about the same. He was also of the opinion that the stronger the tuberculin the fewer non-specific reactions that were obtained.

Buxton (1934) continuing with the preparation of tuberculins on synthetic media remarked on the difference in the tuberculin sensitiveness of cattle as compared with man and other animals, and laid emphasis on the necessity for highly potent tuberculins. In the case of positive animals he found 'old' tuberculin quite satisfactory but difficulties arose owing to non-specific reactions with this tuberculin in negative animals. He stated that these could be overcome by using synthetic media tuberculins. Reference is then made to fluctuations in the sensitivity of the skin in tuberculous animals and he states that in highly allergic animals the injection of even non-specific substances may give rise to a non-specific reaction.

Bull (1936) stresses the importance of a standardised tuberculin as 'old' tuberculin contains non-specific substances which will cause some degree of local reaction

following/....

following intradermal injections in animals free of tuberculosis.

Buxton and Glover (1939) were asked by the Joint Tuberculosis Committee of the Medical and Agricultural Research Councils to submit a report on the methods of manufacture and properties of tuberculin in order to try and obviate difficulties in the interpretation of the double intradermal test. They admit there is no recognised 'standard' tuberculin against which their synthetic tuberculin can be compared and no uniformity in the tests which are applied for the determination of the strength of such tuberculins. In this work they re-affirm their previous views on 'old' tuberculin prepared on broth with regard to positive reactors and stress the likelihood of non-specific reactions when negative animals are tested.

In a leading article in the British Medical Journal of June 17th 1939 the fact that tuberculin for the testing of cattle is not standardised is commented on and it is suggested that the Veterinary experts might attempt this in the same way as the Tuberculin for human use is standardised.

While admitting that all tuberculin for Veterinary use should be standardised, it must not be expected that simply by the use of a standard tuberculin all doubtful reactions will disappear and only clear cut negative or positive reactions will result. This is borne out by articles contributed by medical observers who use a standard tuberculin and there is as yet still not complete agreement as to whether the Mantoux test is superior to the van Pirquet test.

ACID FAST ORGANISMS NOT M. TUBERCULOSIS.

Lydia Rabinowitsch[3] (1897) discovered an acid-fast organism in market butter while studying the subject of tuberculosis in this commodity. This organism when injected into guinea-pigs caused lesions resembling somewhat those of tuberculosis. Further work, however, proved that this organism was not M. tuberculosis. There are a number of these Paratubercle bacilli such as the Smegma bacillus, Bacillus Phlei and Moeller's mist bacillus.

Schroeder (1926) in considering no lesion reactors came to the conclusion that other acid fast organisms not M. tuberculosis might cause sensitisation of animals to tuberculin. In 1932 Hagan and Levine worked on the pathogenicity of the saphrophytic acid fast bacilli. From the description of the lesions in guinea-pigs given these closely simulate the lesions sometimes caused by M. tuberculosis. Holth (1932), as quoted by Plum stated that animals affected with Brucellosis did sometimes give positive reactions to the tuberculin test.

Hastings, Wisnicky, Beach and McCarter (1933) in the study of no lesion reactors, suggest that such reactions may be caused by some other organism or organisms other than bovine M. tuberculosis that may sensitise cattle to tuberculin. They suggest the avian or human type of M. tuberculosis, bacillus of Johne's disease or the saphrophytic members of the acid fast group of organisms.

Schaefer/....

Schaefer (1935) reported on an examination of six cultures of acid-fast bacteria obtained from guinea-pigs. They resembled the avian type of M. tuberculosis but differ from it on serological grounds. The complement-fixation adsorption method carried out on these organisms showed that they possessed components common to both bovine and avian types of M. tuberculosis but by treatment with bovine and avian strains these components can be eliminated leaving a third component which seems to be a specific to each member of this group.

Plum (1937) stated than an animal affected with the avian type or with M. paratuberculosis can also give a positive reaction.

Timoney (1939) remarked on the greater frequency of reactions to the homologous tuberculin as compared with reactions to the heterologus product. The group reactions have been noted by various workers whom he quotes and states they are of the greatest importance to the practitioner who uses the intradermal method of tuberculin testing herds. He suggests it is highly probable that many of the so-called doubtful reactions are due to sensitisation of the bovine tissues by members of the acid fast group of bacteria other than the bovine tubercle bacillus.

Buxton and Glover (1939) carried out a series of experiments on animals by injecting saphrophytic acid fast organisms, non-acid fast organisms and different types of M. tuberculosis. In the case of cattle injected with saphrophytic acid fast organisms no reactions were obtained. Some animals infected artificially with Br. Abortus gave positive reactions when tested with Tuberculin.

In 1934 the writer in an attempt to explain the cause of non-specific reactions experienced in the course of testing animals in the Union of South Africa by means of the double intradermal test carried out experiments in order to find out whethersaphrophytic acid fast organisms, types of M. tuberculosis, Br. abortus, Preiz Nocard organism, and unidentified diphtheroids could cause sensitisation of guinea-pigs to tuberculin prepared from human or bovine strains of M. tuberculosis. These results are now, for the first time, being reported on and form the main portion of this thesis.

The technique of the Double intradermal test, as carried out in the Union, is identical with that carried out overseas, and whether or not the interpretation of so-called reactions differs from overseas interpretations, the fact remains, that lesions of Tuberculosis cannot be found in spite of very careful post-mortem examinations in numbers of cases giving reactions. In many of the cases, lesions of disease are found but they are definitely not caused by the Bacillus of Tuberculosis. Marked reactions have been given in animals that showed extensive abscesses of the liver, in animals the subject of Contagious Abortion and Actinomycosis and finally in animals whose post-mortem was negative and yet were close to parturition.

It was suggested that these animals harboured acid-fast organisms, not the specific Tuberculosis organism, and that these were the cause of the apparent positive Tuberculosis reactions.

Cases have been observed in two cows where reactions were almost identical in extent and in character, i.e. oedema, heat, tenderness, etc., and yet on post-mortem the one showed characteristic lesions of Tuberculosis while the other was a case of extensive liver abscess.

I have to thank Drs. Robinson and de Kock for suggesting this work, for encouragement and for the interpretation of many of the results obtained.

METHOD: The first group of guinea pigs were infected artificially with acid-fast organisms of the Tuberculosis group while others were injected with Bacillus Abortus bovis and strains of various diphtheroid organisms.

The second group was infected with organisms of the acid-fast type not belonging to the Tuberculosis organism group, the Preiz Nocard organism and a strain of C.Pyogenes.

In.../

In order to sensitise the guinea pigs for testing, emulsions of various acid-fast organisms were made up as follows:-

(a) Young growing cultures of the organisms were washed off with sterile saline solution.

(b) Young growing cultures were made up in oily suspensions of Liquid Paraffin according to the method used by Hagan and Levine, (1932). Their method was to wash off the growths of the organisms into small bottles containing a few cubic centimetres of sterile salt solution and a few glass beads. These were shaken vigorously in a shaking machine for a short time to ensure a fairly uniform suspension being obtained. The suspensions were now poured into tubes and diluted with more saline until the density of all was about uniform. Ten cubic centimetres portions were placed in centrifuge tubes and the organisms were thoroughly sedimented. The fluid was now drained off and replaced by the same amount of sterile mineral oil. These mixtures were now subjected to prolonged shaking in order to incorporate the organisms in the oil. The final suspensions were milky white and fairly stable.

Of these various emulsions, 1 cubic centimetre was taken and injected either subcutaneously or by the intraperitoneal route, the object of this being to see which of these methods would cause the greater infection, and be followed by a possible reaction to substances used in testing.

In the first experiment 48 guinea pigs were used and were divided up as follows:-

Cage.../

Page	Date of Inoculation	No. of Guinea Pigs used	Channel of Artificial Infection	Emulsion used and Amount
A	5/6/33	(Six	Subcutaneous	Saline emulsion of B.C.G. 1 c.c.
		(Two	Controls	Controls.
B	"	(Six	Intraperitoneal	Saline emulsion of B.C.G. 1 c.c.
		(Two	Controls	Controls.
C	"	(Six	Subcutaneous	Saline emulsion of M.Phlei 1 c.c.
		(Two	Controls	Controls.
D	"	(Six	Subcutaneous	Saline emulsion of Avian M.Tuberculosis 1 c.c.
		(Two	Controls	Controls.
E	"	(Six	Subcutaneous	Liquid paraffin and M.Phlei 1 c.c.
		(Two	Controls	Controls.
F	"	(Six	Subcutaneous	Liquid paraffin and Avian M.Tuberculosis 1 c.c.
		(Two	Controls	Controls.

Each pair of controls were placed in contact in the same cages as their respective group of inoculated Guinea Pigs.

Twenty-four days after inoculation they were tested by the intradermal Tuberculin Test using Standard Tuberculin in dilutions varying from $\frac{1}{500}$ to $\frac{1}{4000}$ viz. $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.

Reactions were classed as follows:-

— Negative, no redding of skin at site of test.

+— Doubtful, very slight reddening of skin at site of test.

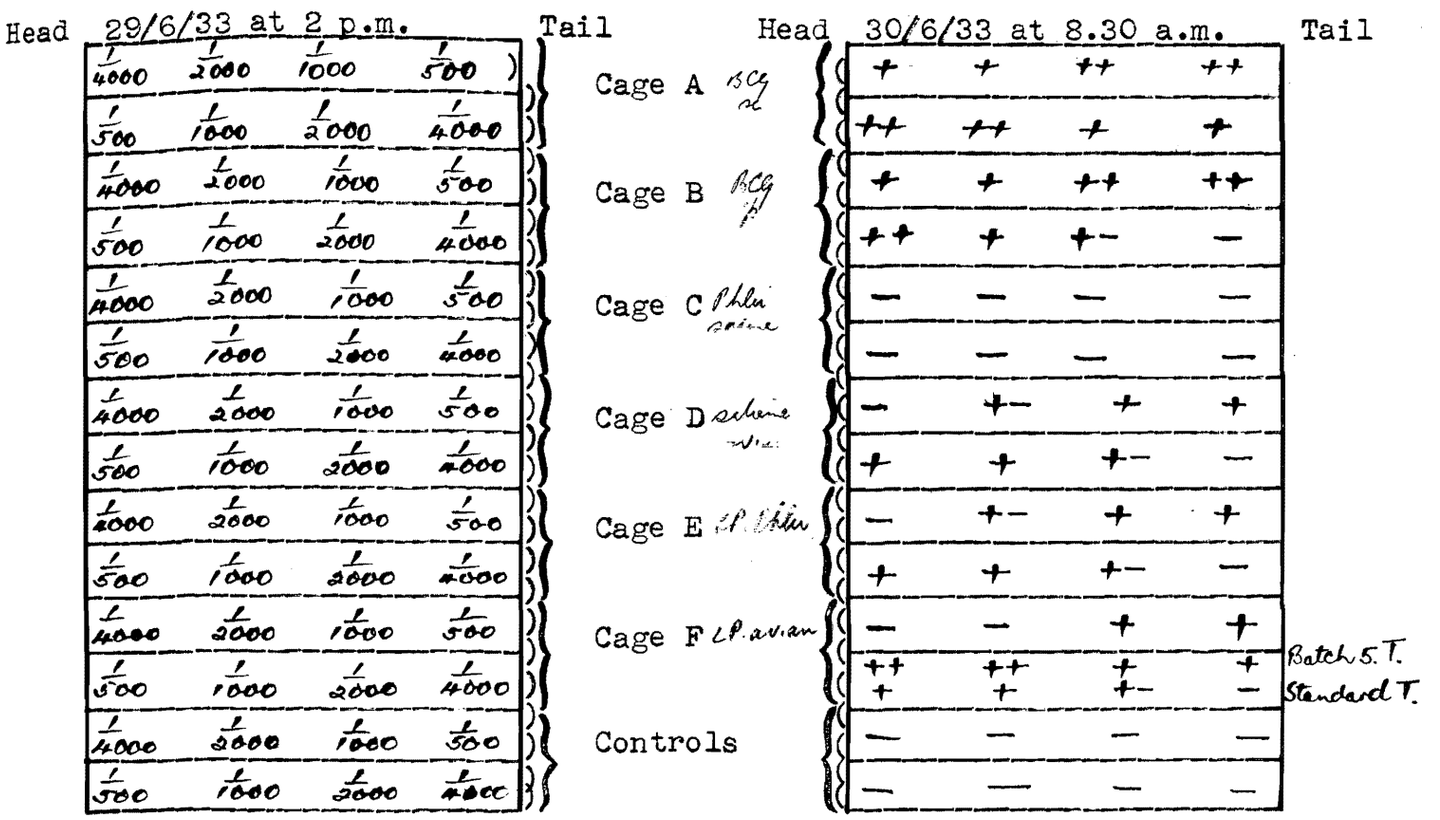
\+ Positive, distinct reddening of skin at site of test.

++ Marked positive, very marked reddening of skin at site of test, in some cases a commencing necrosis in centre of red area.

Two guinea pigs from each group were used at each test.

Date 29/6/33 - 24 days after inoculation.

Tested with Standard Tuberculin in dilutions $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$ injected intradermally.



Reactions in Subcutaneous B.C.G. inoc. G.pigs (marked)

Reactions in Intraperitoneal B.C.G. inoc. G.pigs (marked)

Reactions in Subcutaneous Avian M.Tuberculosis (Saline) inoc. G.pigs (slight)

Reactions in Subcutaneous M.Phlei + liquid paraffin inoc. G.pigs (slight)

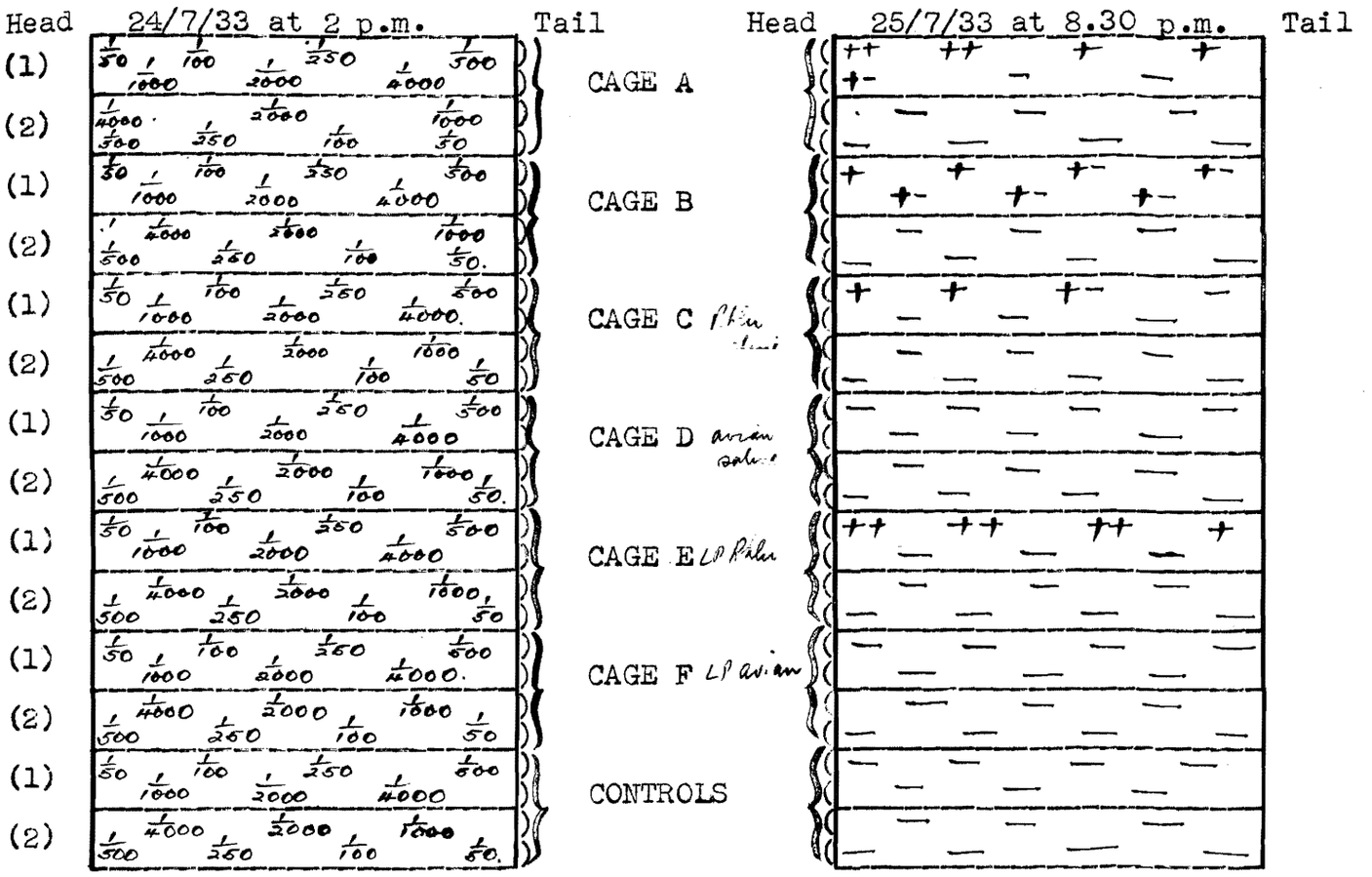
Reactions in Subcutaneous Avian M.Tuberculosis + liquid paraffin inoc. G.pigs (Slight)

As a result of these reactions it was decided to use stronger solutions of Tuberculin and consequently the dilutions used were as follows:- $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.

21.../

Date 24/7/33. Second Intradermal test of guinea pigs that were tested on 29/6/33.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.



Reaction in guinea pig injected subcutaneously with B.C.G. in one Case.

Reaction in guinea pig injected intraperitoneally with B.C.G. in one Case.

Slight reaction in guinea pig injected subcutaneously with M.Phlei in one Case.

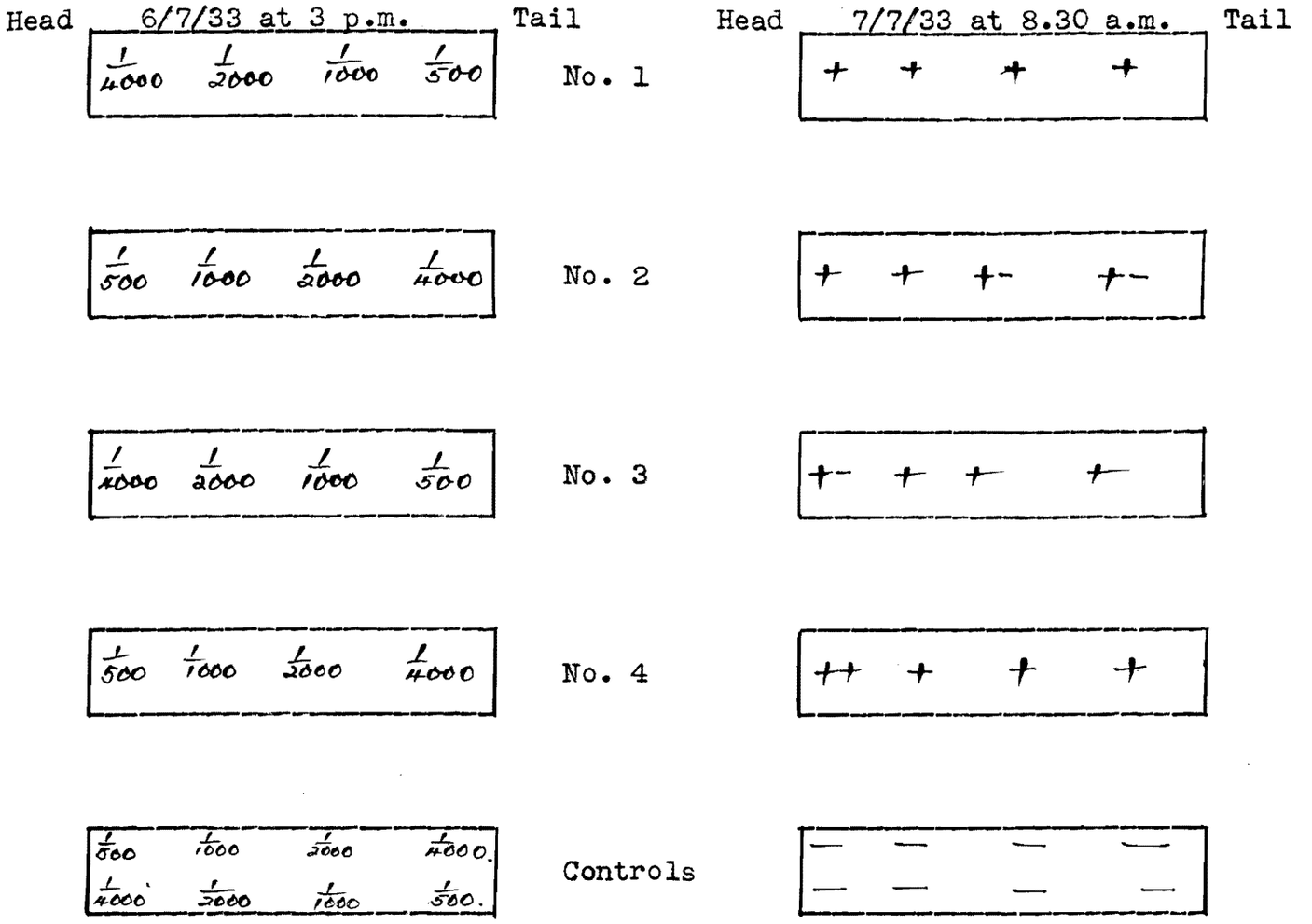
Reaction in guinea pig injected subcutaneously with M.Phlei & oil in one Case.

While doing these tests a number of Guinea Pigs that had been injected intraperitoneally with 1 c.c. of Contagious Abortion organisms in Saline became available so they were included.

22.../

DATE 6/7/33.

Tuberculin intradermal test on guinea pigs injected with Contagious Abortion emulsion on 4/10/32. CAGE 126.



Tuberculin used: Batch 5 in dilution No. $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.

The Tuberculin was prepared at the Laboratory here and compared favourably with Standard Tuberculin as the result of the tests.

Reactions given in all cases.

These guinea pigs were now transferred to cages 195 and 196.

On 10/7/33 - 35 days after inoculation, a further test was carried out on two more guinea pigs from each group and on 11/7/33 the remaining two guinea pigs of each group were tested.

23.../

DATE 10/7/33 - 35 days after inoculation.

Tested with Standard Tuberculin in Dilutions $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.

10/7/33 at 2 p.m.					11/7/33 at 8.30 a.m.			
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CAGE A	—	—	—	—
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$		—	—	—	—
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CAGE B	—	—	+–	+–
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$		+	+	+	+
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CAGE C	—	—	—	—
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	Phlei saline	—	—	—	—
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CAGE D	—	—	+–	+–
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	avian saline	+–	+–	—	—
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CAGE E	+–	+–	+	+
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	L.P. Phlei	—	—	—	—
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CAGE F	—	—	+–	+
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$	L. Avian	+	+–	—	—
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	Contagious Abortion Cage	+–	+–	+	+
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$		+–	+–	+–	—
$\frac{1}{4000}$	$\frac{1}{2000}$	$\frac{1}{1000}$	$\frac{1}{500}$	CONTROLS	—	—	—	—
$\frac{1}{500}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{4000}$		—	—	—	—

Contagious Abortion injected guinea pigs from Cage 124, injected with Bacillus Abortus 19/9/32.

Reactions were given in the guinea pigs infected

(a) Intraperitoneally with B.C.G. strain of T.B.

Slight reactions were given in guinea pigs infected by the subcutaneous route with -

(a) Avian M.tuberculosis strain;
(b) M.Phlei + L.Paraffin;
(c) Avian M.tuberculosis strain + L.Paraffin;
(d) Br.abortus.

A further Tuberculin test was done on these Contagious Abortion injected guinea pigs on 31/7/33 using smaller and more dilutions of Tuberculin.

24.../

Date 11/7/33 - 36 days after inoculation.

Tested with Standard Tuberculin in dilutions $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$.

Head 11/7/33 at 2 p.m.				Tail	Head 12/7/33 at 8.30 a.m.				Tail
1/50	1/100	1/250	1/500	CAGE A	+	+	+	+	
1/500	1/250	1/100	1/50		+	+	++	++	
1/50	1/100	1/250	1/500	CAGE B	++	++	+	+	
1/500	1/250	1/100	1/50		+	+	+	++	
1/50	1/100	1/250	1/500	CAGE C Phlei control	—	—	—	—	
1/500	1/250	1/100	1/50		—	—	—	—	
1/50	1/100	1/250	1/500	CAGE D avian control	+	+	+	+-	
1/500	1/250	1/100	1/50		—	+-	+	+	
1/50	1/100	1/250	1/500	CAGE E Lp phlei	+	+	+-	+-	
1/500	1/250	1/100	1/500		+	+	++	++	
1/50	1/100	1/250	1/500	CAGE F Lp avian	+	+	+-	—	
1/500	1/250	1/100	1/50		+-	+-	+	+	
1/50	1/100	1/250	1/500	Contagious Abortion Cage	+	+-	—	—	
1/500	1/250	1/100	1/50		—	—	—	—	
1/50	1/100	1/250	1/500	T.B. CAGE	++	++	++	+	
1/500	1/250	1/100	1/50		+*	+	++	++	
1/50	1/100	1/250	1/500	CONTROLS	—	—	—	—	
1/500	1/250	1/100	1/50		—	—	—	—	

2nd Guinea Pig of control killed, all organs normal, guinea pig pregnant. Guinea pigs of C.A. cage inoculated 31/10/32, from cage 130.

T.B. Cage - Guinea Pigs inoculated 1 month previous with T.100 (human strain).

Slight reactions were obtained in guinea pigs inoculated -
(a) Subcutaneously with Saline emulsion of avian M.tuberculosis.
(b) Subcutaneously with L.Paraffin emulsion of avian M.tuberculosis.

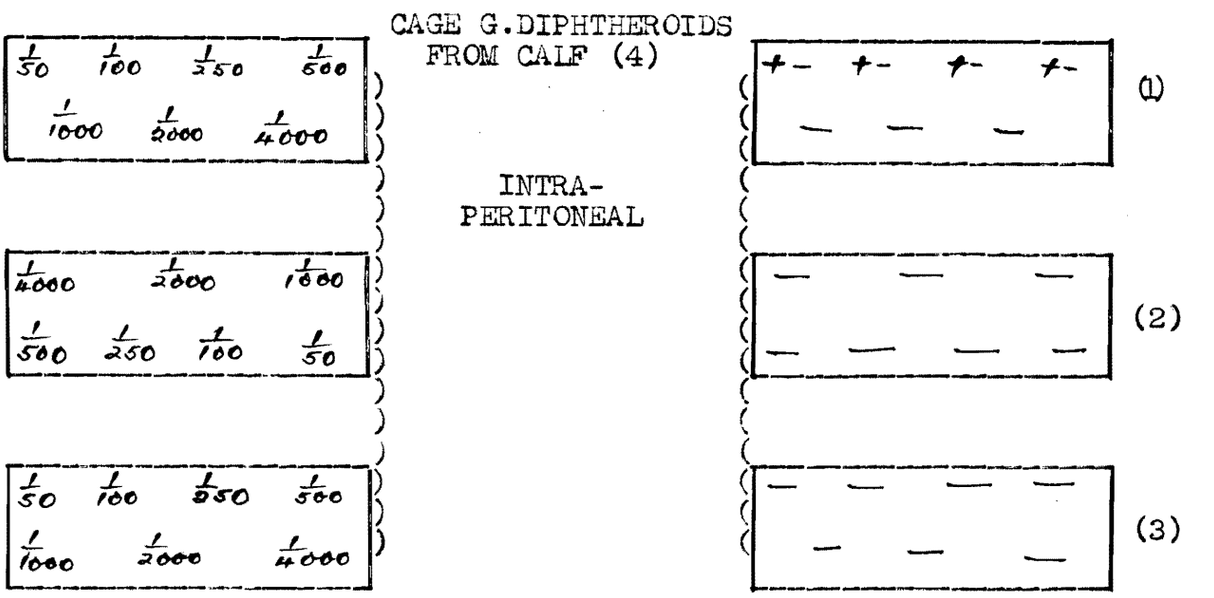
Moderate reactions were obtained in guinea pigs inoculated -
(a) Subcutaneously with Saline emulsion of B.C.G. strain of M.tuberculosis.
(b) Subcutaneously with L.Paraffin emulsion of M.Phlei.

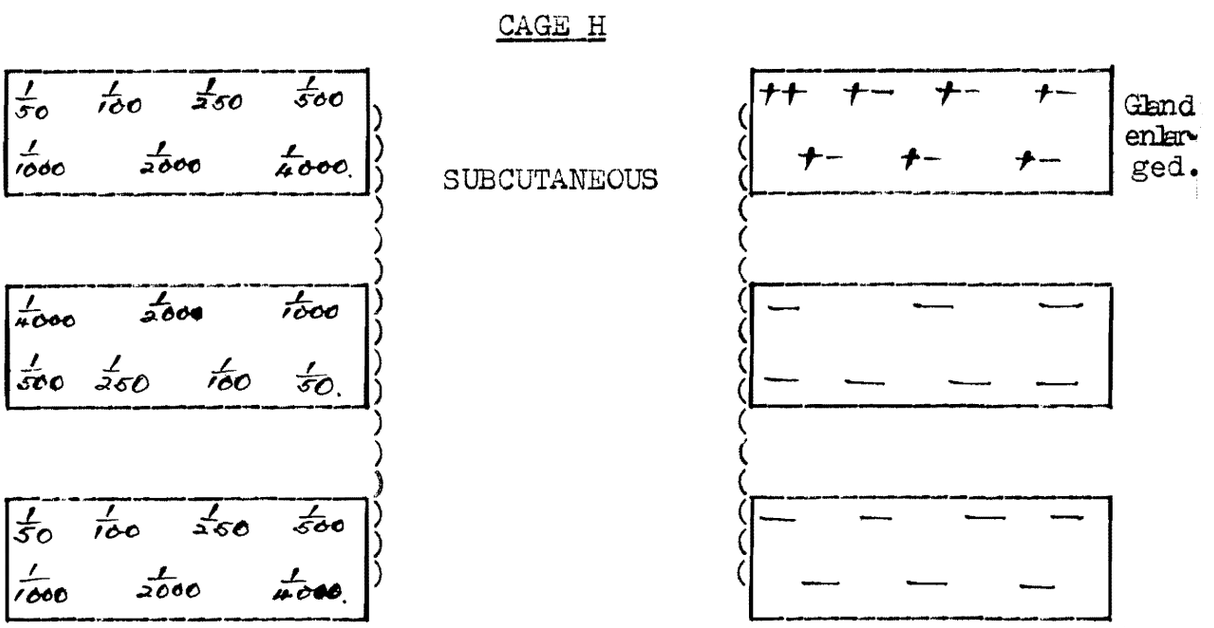
Marked reactions were obtained in guinea pigs inoculated -
(a) Intraperitoneally with Saline emulsion of B.C.G. strain of M.tuberculosis.
(b) Intraperitoneally with Saline emulsion of human strain of M.tuberculosis.

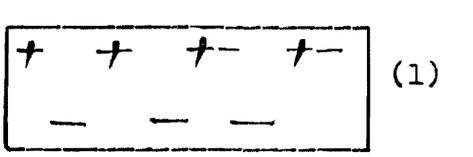
25.../

Date 21/8/33. Inoculated on 25/7/33.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.







This reading was made 48 hours after test.

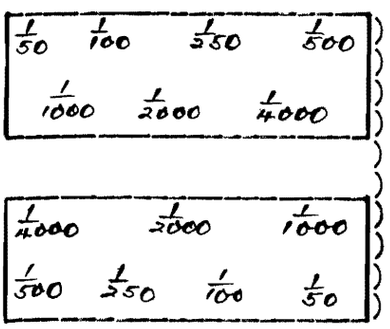
Slight reaction in two guinea pigs.

26.../

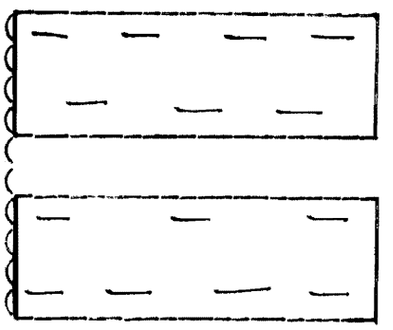
DATE 21/8/33 - Inoculated on 25/7/33.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE I



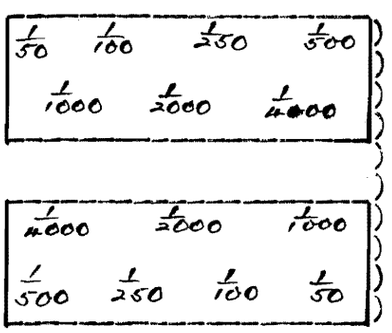
Supra Mammary Culture of Diphtheroids. Subcutaneous.



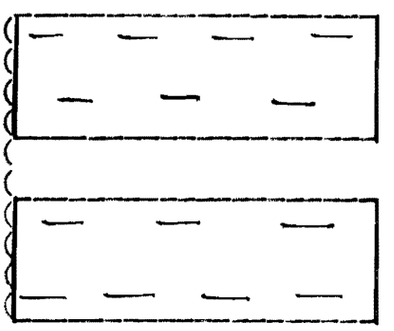
Results - Negative

DIPHTHEROIDS

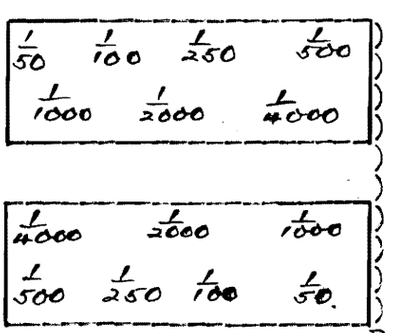
CAGE J



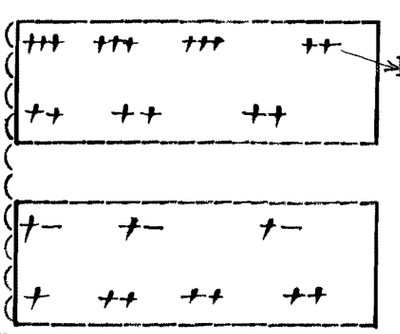
Supra Mammary Culture of Diphtheroids. Intraperitoneal.



Results - Negative

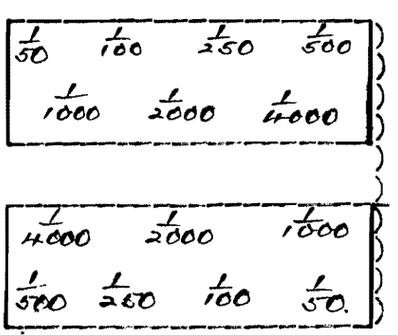


T.100 strain of M.Tuberculosis.

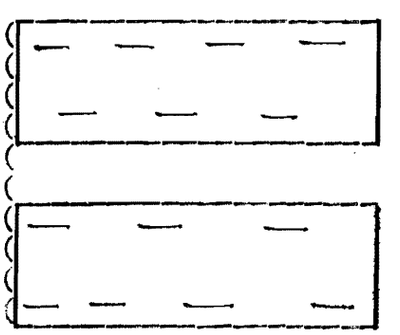


Necrosis.

Results - Marked reactions



C O N T R O L S

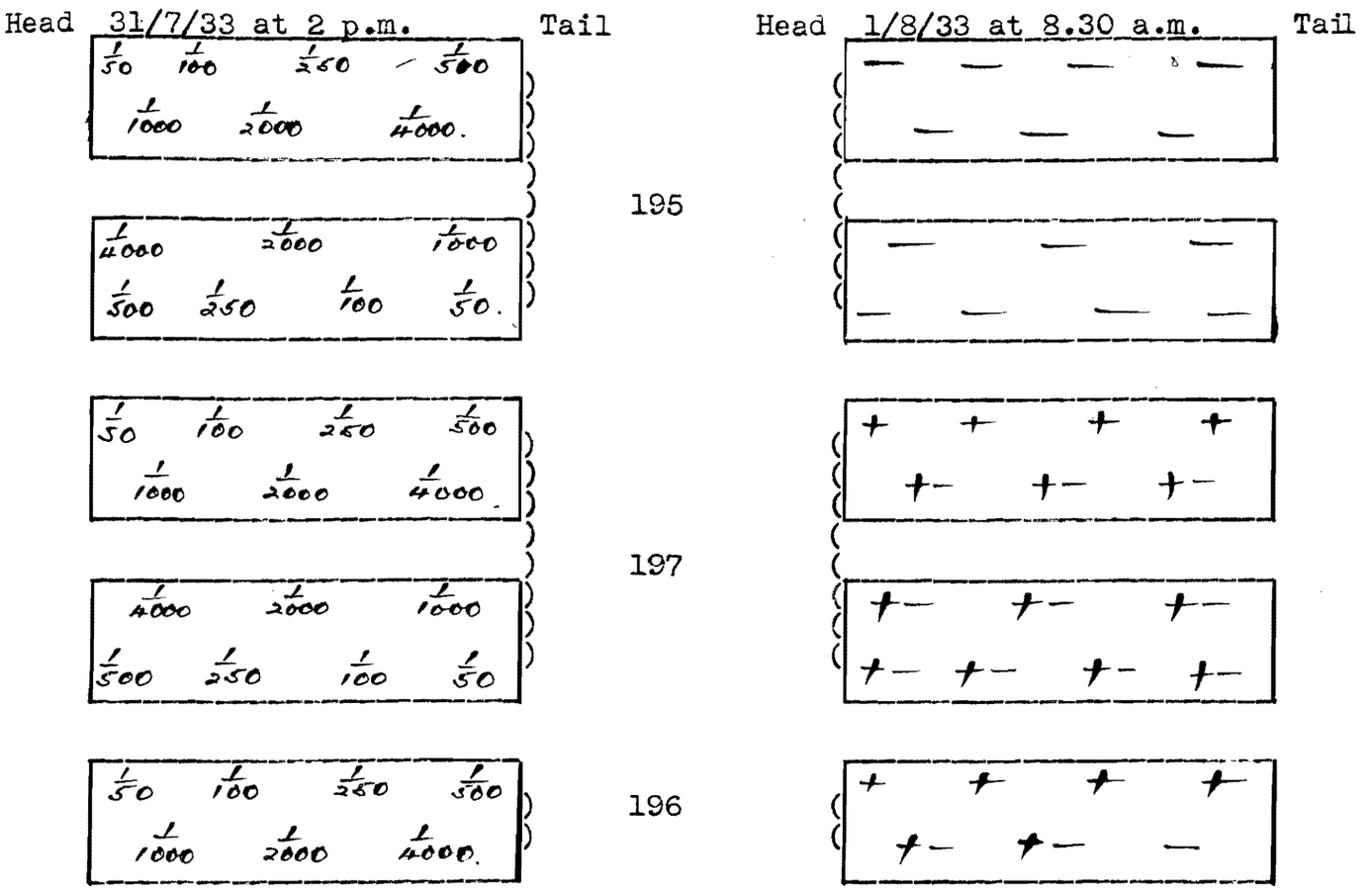


Results - Negative

27.../

DATE 31/7/33.

Tuberculin intradermal test on Guinea Pigs injected with Contagious Abortion emulsion, viz. cages 195, 196, 197.



Controls over page.

Cage 195 - Guinea Pigs with C.A. emulsion intraperitoneal 4/10/32.

Cage 196 - Guinea Pigs with C.A. emulsion intraperitoneal 4/10/32.

Cage 197 - Guinea Pigs with C.A. emulsion intraperitoneal 19/9/32.

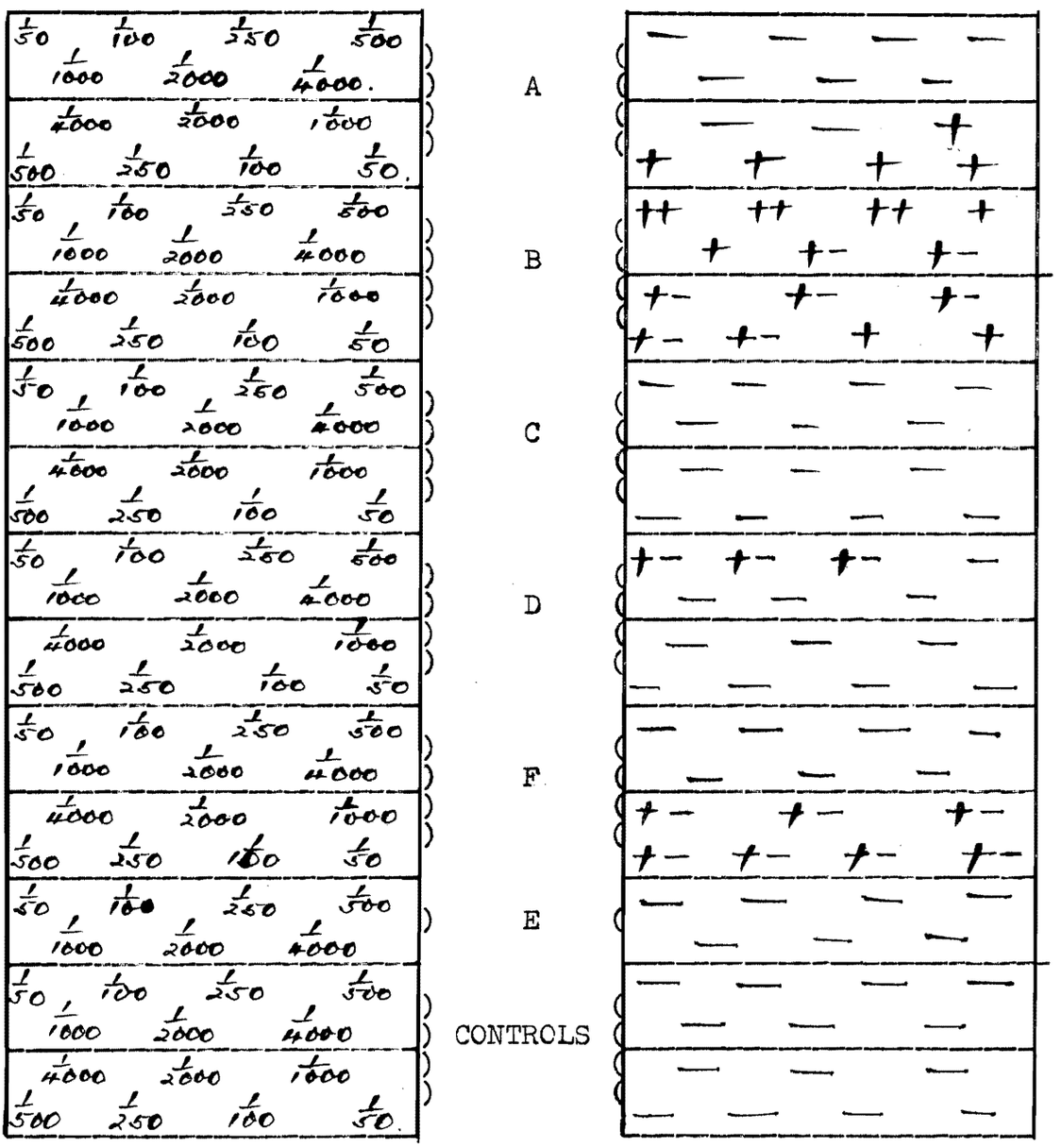
Reactions - slight- were given by Guinea Pigs from Cages 196 & 197.

To close up this experiment two guinea pigs already tested were taken from each group and retested. This was on 31/7/33 - 56 days after inoculation.

28.../

DATE 31/7/33.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.



Guinea Pig, 2nd of E, died 31/7/33 - see end.

Moderate reactions given in guinea pigs inoculated -

(a) Subcutaneously with saline emulsion of B.C.G. strain of M.tuberculosis.

(b) Intraperitoneally with saline eumulsion of B.C.G. strain of M.tuberculosis.

Doubtful reactions given in guinea pigs inoculated -

(a) Subcutaneously with saline emulsion of avian type of M.tuberculosis.

(b) Subcutaneously with L.paraffin emulsion of avian type of M.tuberculosis.

29.../

SUMMARY OF RESULTS:

24 Days after being infected, guinea pigs inoculated with Saline emulsions of the B.C.G. strain of M.Tuberculosis, both by subcutaneous and intraperitoneal channels, showed marked local reactions to Tuberculin injected intradermally. Slight local reactions were shown in guinea pigs inoculated subcutaneously with Saline emulsions of the Avian type of M.Tuberculosis, liquid paraffin emulsion of the same organism and liquid paraffin emulsion of M.Phlei.

Guinea pigs inoculated by the subcutaneous route with Saline emulsions of M.Phlei, gave no local reactions to the test with Tuberculin.

The highest dilution at which the Tuberculin gave marked reactions was 1 - 1000.

49 Days after being infected, local reactions to Tuberculin were observed in one case of subcutaneously inoculated guinea pigs with a Saline emulsion of B.C.G. strain of M.Tuberculosis, the highest dilution now being only 1 - 100. A similar reaction, but going up to a dilution of 1 - 250, was observed in one guinea pig inoculated subcutaneously with a liquid paraffin emulsion of M. Phlei. Slight, but definite tapering reactions up to 1 - 4000, were seen in the guinea pigs inoculated intraperitoneally with a Saline emulsion of the B.C.G. strain of M.Tuberculosis.

Guinea pigs inoculated subcutaneously with an emulsion of Br.abortus organisms, also gave definite reactions.

35 Days after inoculation guinea pigs inoculated intraperitoneally with a Saline emulsion of B.C.G. strain gave definite reactions to dilutions of from $\frac{1}{500}$ to $\frac{1}{4000}$ of Tuberculin.

Varying.../

Varying, very slight reactions, in the small dilutions of Tuberculin, were seen in guinea pigs -

(a) Subcutaneously inoculated with Saline emulsion of Avian T.B. Strain.
(b) Subcutaneously inoculated with Liquid Paraffin emulsion of Avian T.B. Strain.
(c) Subcutaneously inoculated with Liquid Paraffin emulsion of M.Phlei.
(d) Subcutaneously inoculated with Saline emulsion of Br.abortus organisms.

After 36 days, another batch of similarly infected guinea pigs were tested, with identical, but more marked reactions. Guinea pigs inoculated intramuscularly with 1 m.g. of a human strain of the M.Tuberculosis gave very marked results. In this batch, however, the dilutions of Tuberculin used varied from $\frac{1}{50}$ to $\frac{1}{500}$. An interesting result was noted in one of the control guinea pigs. This animal gave slight but definite reactions to all dilutions of Tuberculin. It was destroyed and post-mortem revealed almost full-time young in utero. This is of importance, because, some cows tested with Tuberculin, when near to calving, may give suspicious positive reactions, which are not present, when retested, some time after parturition.

Results after 56 days inoculation, resembled those tested after 49 days inoculation, mentioned earlier in the summary.

In view of the results obtained in the first portion of this work, it was decided to carry out further experiments using Acid-fast organisms, not M.Tuberculosis, the Preiz-Nocard bacillus and a strain of C.pyogenes.

The guinea pigs were inoculated in the same way as in the previous experiment, but, in every case, the vehicle used for suspending the organisms for inoculation was liquid paraffin.

Forty-two guinea pigs with controls were used, and they were divided up as follows:-

date.../

Date of Inoculation - 1/8/33.

No.of Cage	No. of Pigs Used	Channel of Artificial Infection	Emulsion used and Amount
1	3	Subcutaneous	Liquid Paraffin + M.Phlei 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + M.Phlei 1 c.c.
	3	Controls	Controls
2	3	Subcutaneous	Liquid Paraffin + M.Rabinowitz 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + M.Rabinowitz 1 c.c.
	3	Controls	Controls
3	3	Subcutaneous	Liquid Paraffin + M.Smegmatis 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + M.Smegmatis 1 c.c.
	3	Controls	Controls
4	3	Subcutaneous	Liquid Paraffin + Moeller's Mist Bac. 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + Moeller's Mist Bac. 1 c.c.
	3	Controls	Controls
5	3	Subcutaneous	Liquid Paraffin + C.P.Nocard (horse) 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + C.P.Nocard (horse) 1 c.c.
		Controls	Controls
6	3	Subcutaneous	Liquid Paraffin + C.P.Nocard (sheep) 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + C.P.Nocard (sheep) 1 c.c.
	3	Controls	Controls
7	3	Subcutaneous	Liquid Paraffin + C.Pyogenes 1 c.c.
	3	Intraperitoneal	Liquid Paraffin + C.Pyogenes 1 c.c.
	3	Controls	Controls

Controls were placed in the same cages as their respective groups of inoculated Guinea Pigs.

In from 20 to 21 days after inoculation, these Guinea Pigs were tested out with Standard Tuberculin in dilutions of $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$.

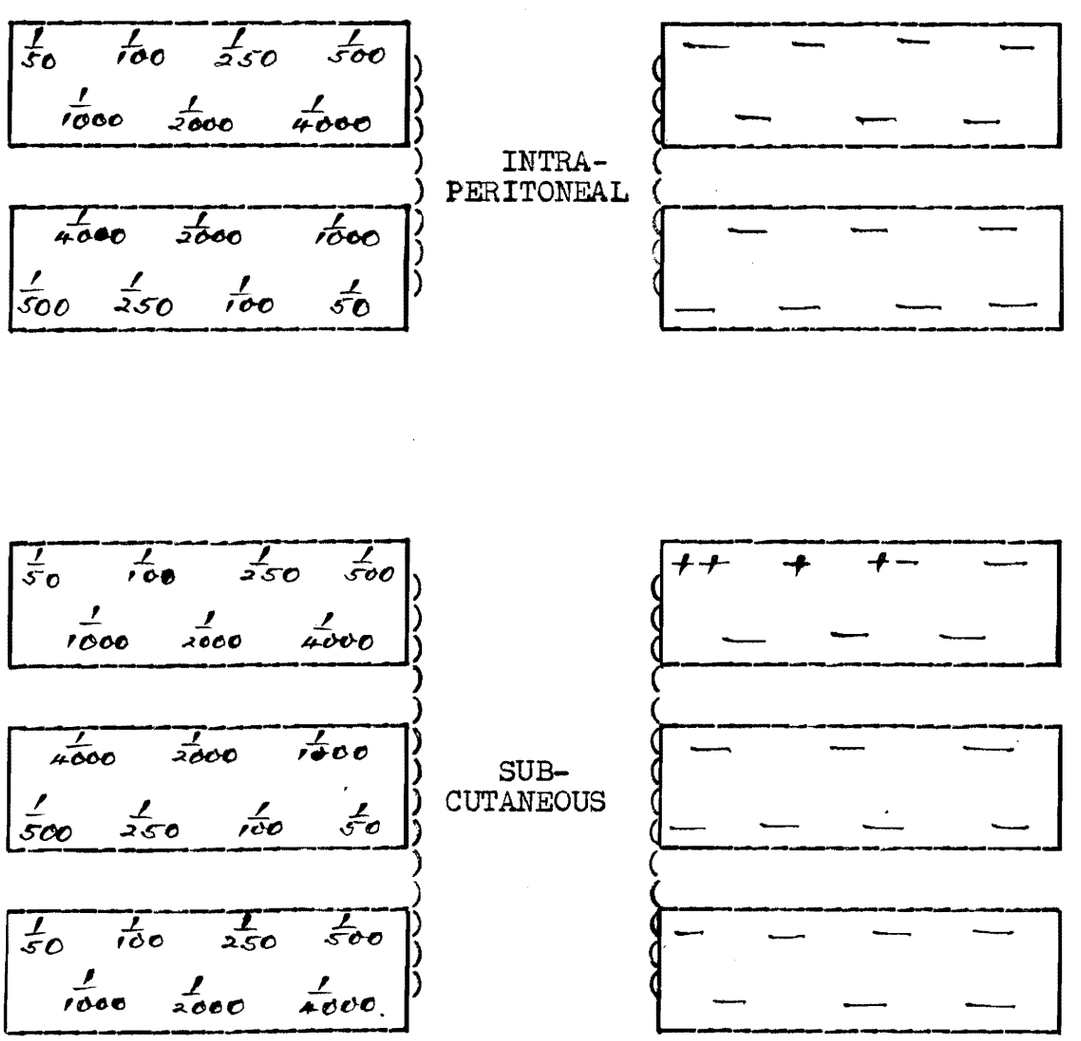
Reactions were classed as in previous experiment.

32.../

Date 22/8/33 - 21 days after inoculation.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE 1. M.PHLEI



RESULTS:

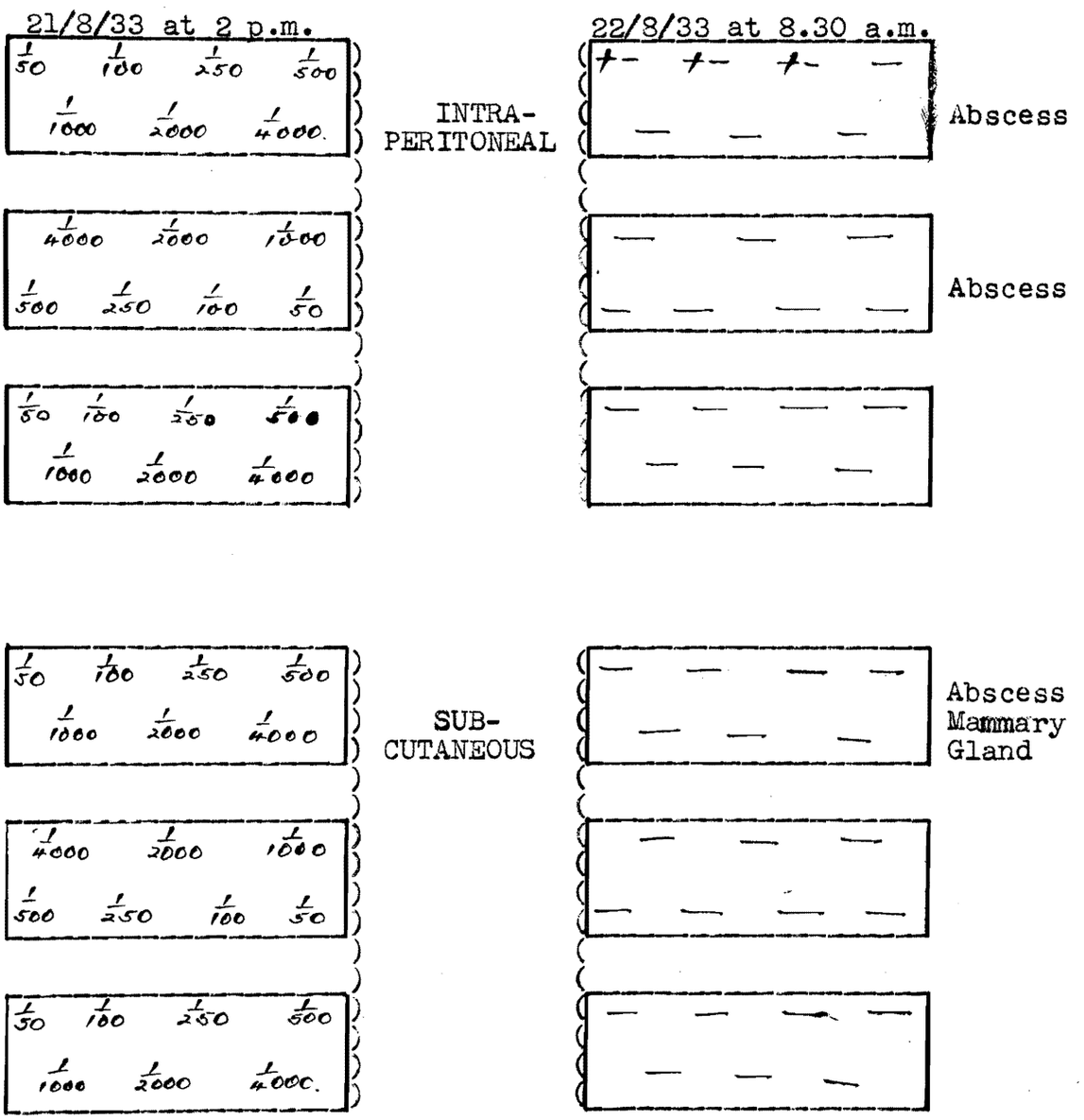
Slight reaction in one guinea pig of subcutaneously inoculated group. Controls tested same time, negative.

33.../

DATE 21/8/33 - 20 days after inoculation.

Tested with Standard Tuberculin $\frac{1}{50}$, $\frac{1}{100}$, $\frac{1}{250}$, $\frac{1}{500}$, $\frac{1}{1000}$, $\frac{1}{2000}$, $\frac{1}{4000}$, dilutions.

CAGE 2. M.RABINOWITZ
+ LIQUID PARAFFIN



RESULTS:

Very light reaction in one Intraperitoneally inoculated guinea pig. Two of these showed abscess formation extending along track of needle.

In the Subcutaneously inoculated group, one showed a large abscess in mammary gland.

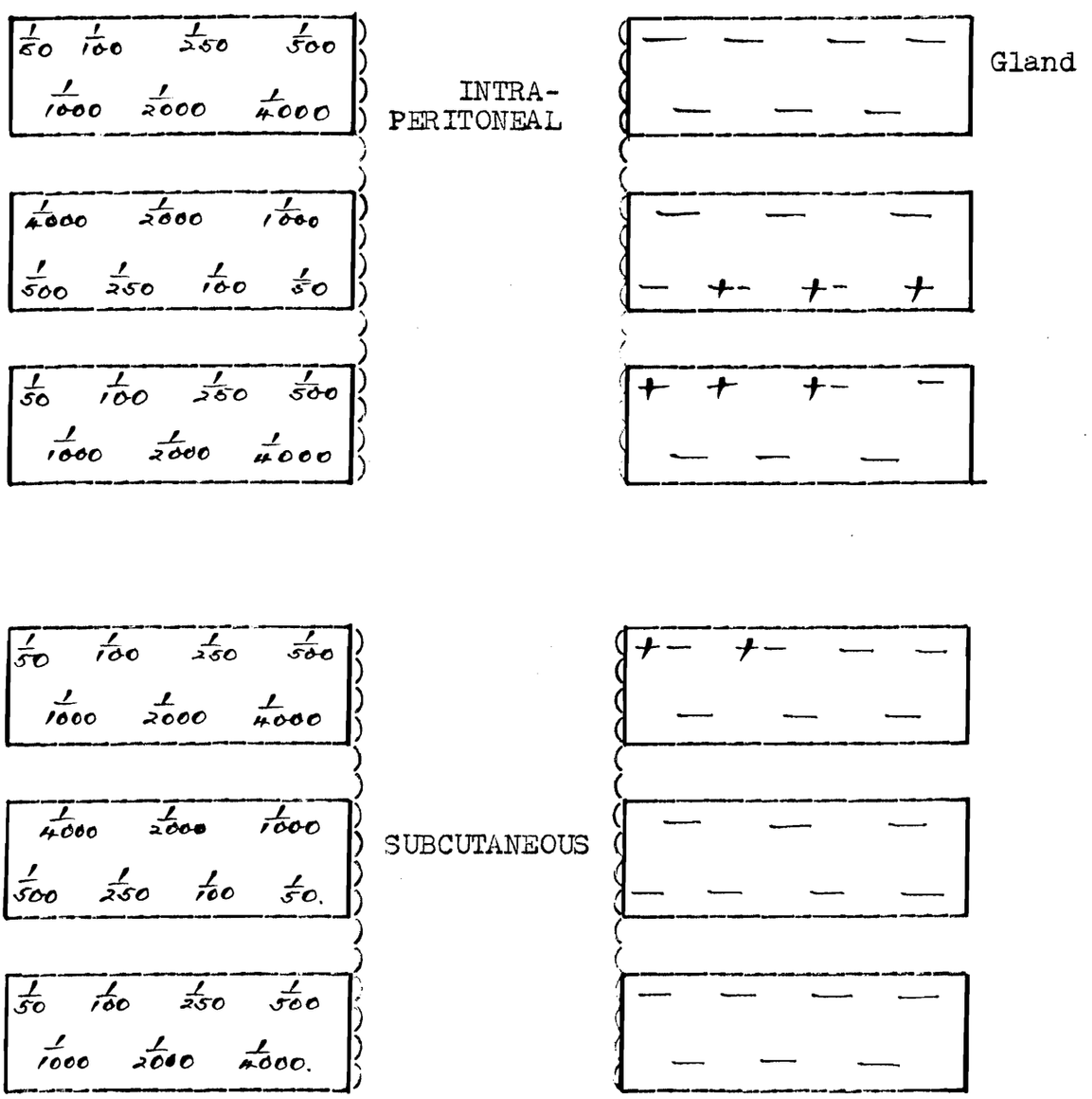
Controls negative.

34.../

DATE 22/8/33 - 21 days after inoculation.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE 3. M.SMEGMATIS



RESULTS:

Two guinea pigs of the Intraperitoneal inoculated group showed slight reactions in the smallest dilutions. One of these, giving no reactions, had an enlarged Inguinal gland. One guinea pig of subcutaneous inoculated group showed slight suspicious reactions.

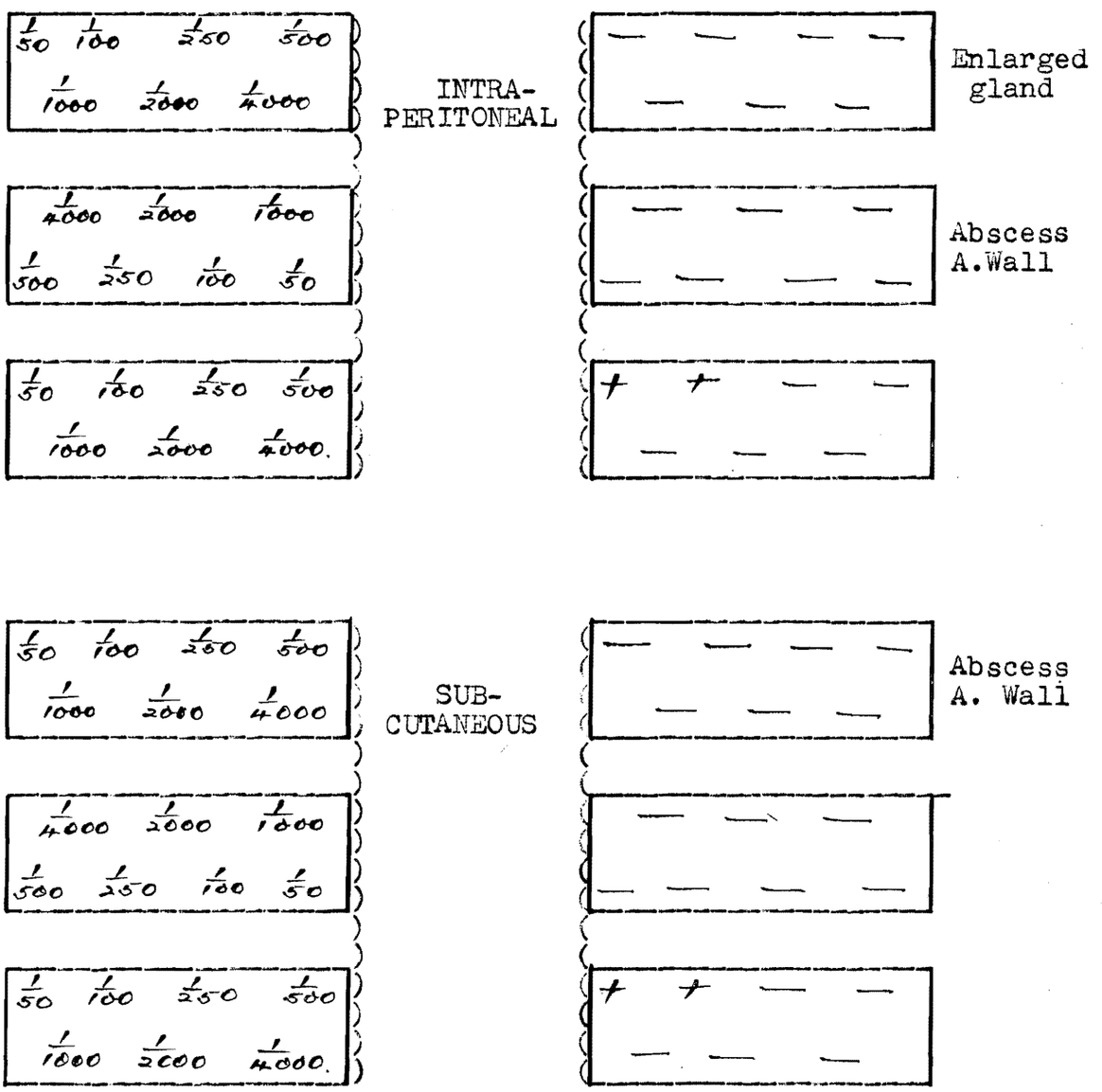
Controls negative.

35.../

DATE 21/8/33 - 20 days after inoculation.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE 4. MIST BACILLUS
+ LIQUID PARAFFIN



RESULTS:

One guinea pig of Intraperitoneal inoculated group showed slight reactions. One of the two non-reactors of this group, had a very much enlarged Inguinal gland, while the other, had an abscess of the abdominal wall, along where needle entered. One guinea pig of Subcutaneous inoculated group, showed a slight reaction, while one of the two non-reacting guinea pigs, had an abscess at seat of inoculation.

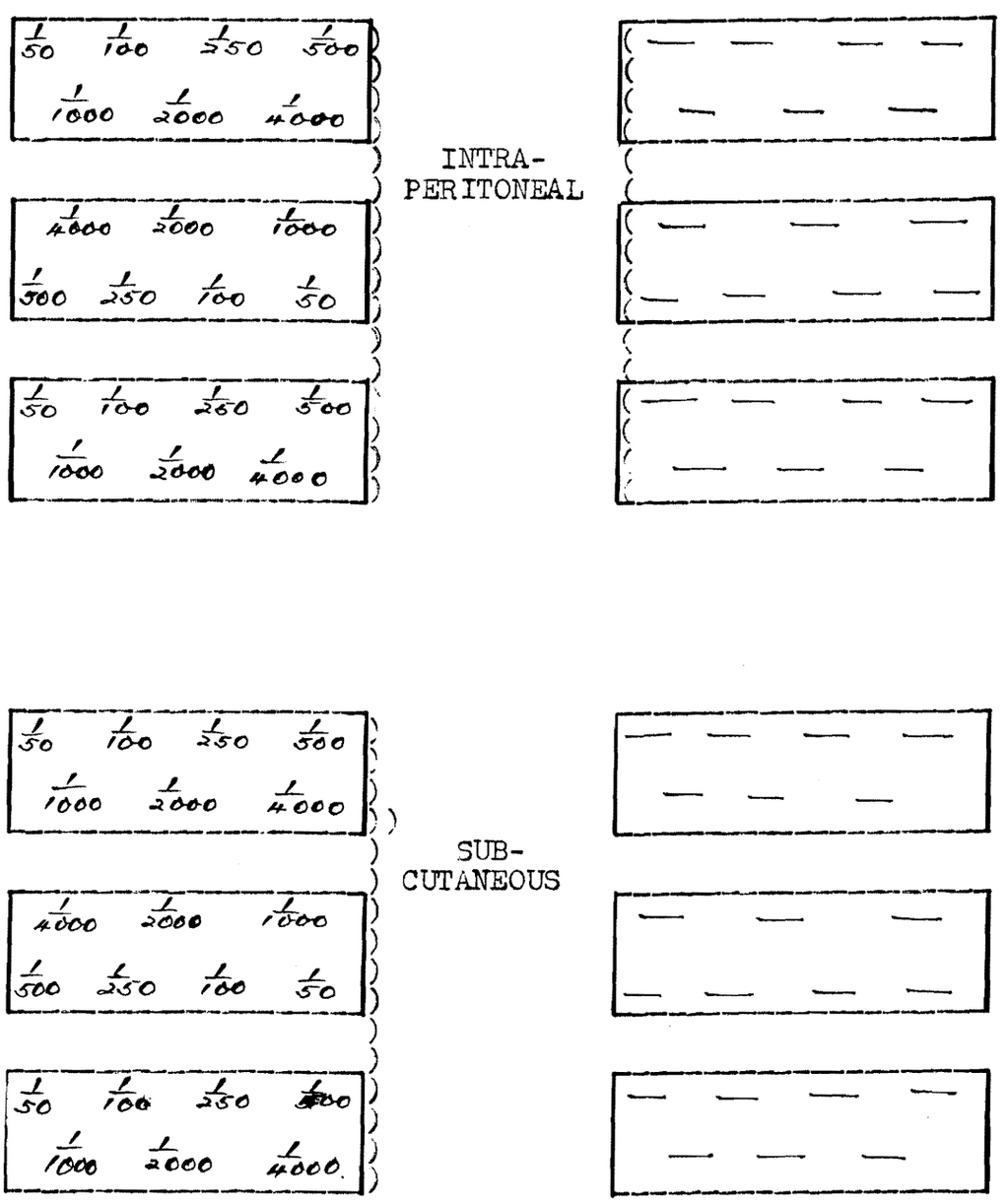
Controls negative.

36.../

DATE 21/8/33 - 20 days after inoculation.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE 5. C.P.NOCARD
(HORSE)



RESULTS:

No reactions.

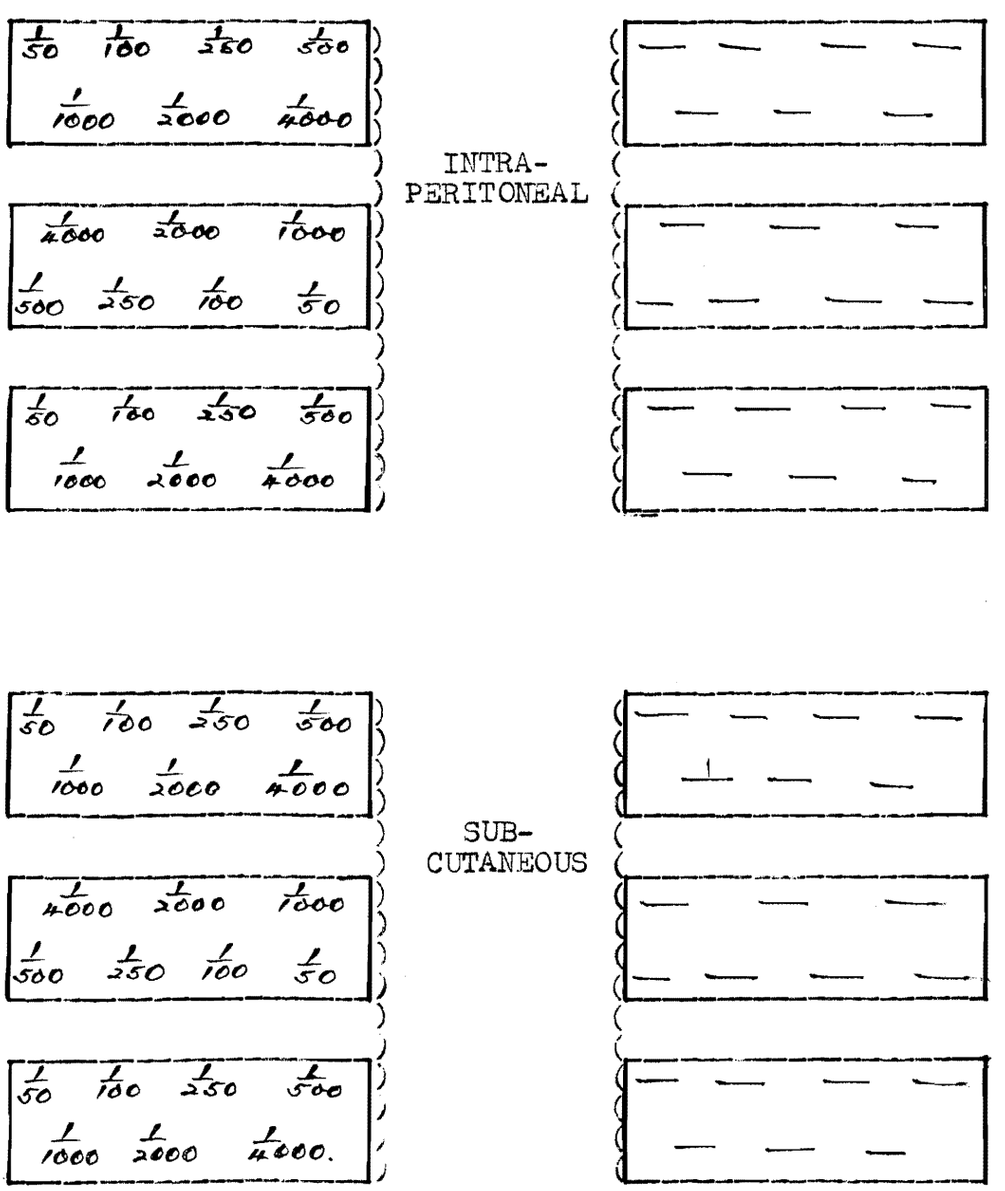
Controls negative.

37.../

DATE 21/8/33 - 20 days after inoculation.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE 6. C.P.NOCARD
(SHEEP)



RESULTS:

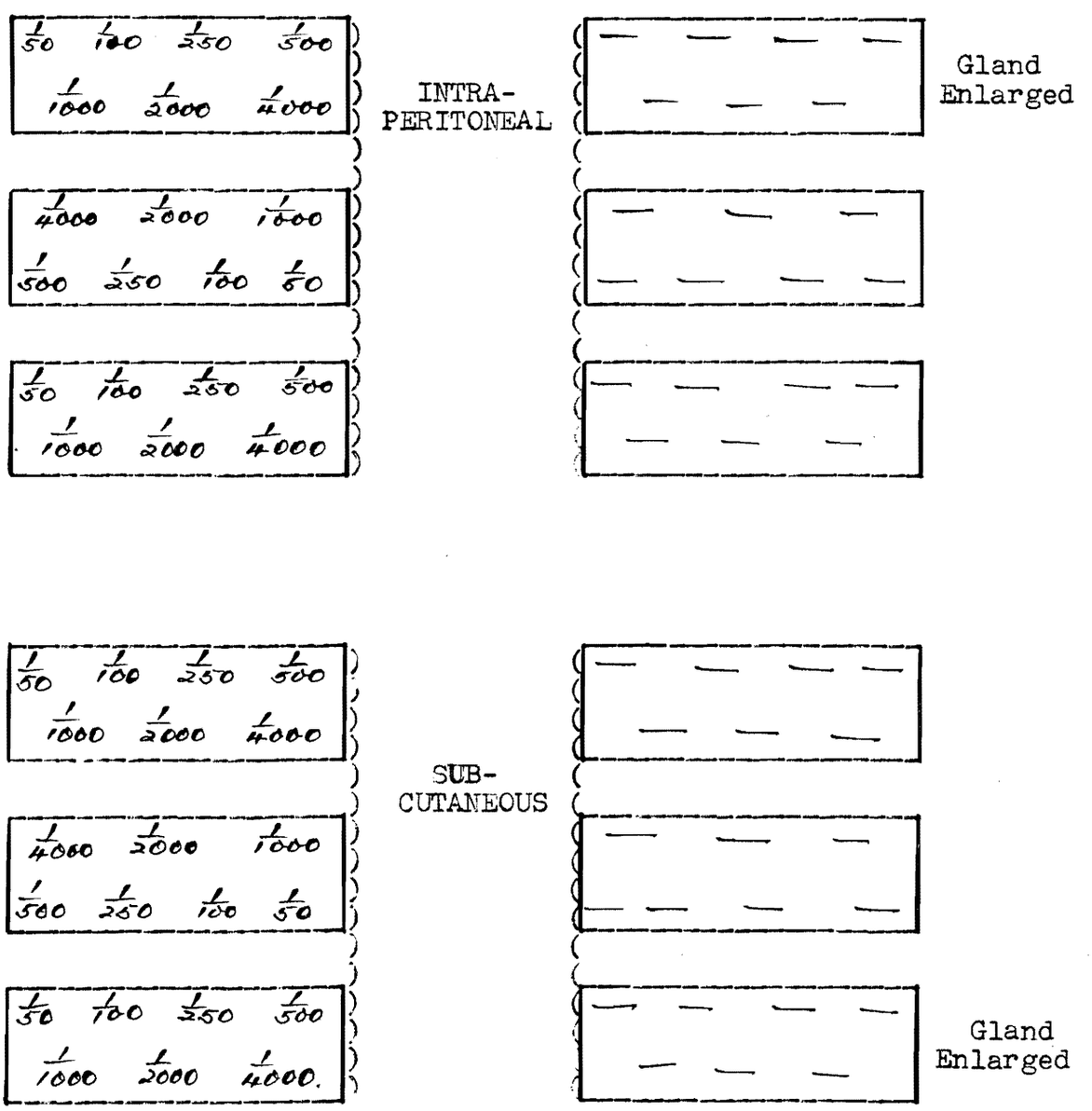
No reactions.
Controls negative.

38.../

DATE 21/8/33 - 20 days after inoculation.

Tested with Standard Tuberculin in dilutions of $\frac{1}{50}$ to $\frac{1}{4000}$.

CAGE 7. C.PYOGENES



RESULTS:

No reactions.

Controls negative.

39.../

SUMMARY:

This experiment revealed the fact that these guinea pigs, although affected with acid-fast organisms, not M.Tuberculosis, did not give reactions when tested with Standard Tuberculin by the Intradermal method.

Having had no reactions with Tuberculin it was decided to prepare so-called 'Tuberculins' or Extracts from the organisms with which the guinea pigs were infected.

The method of preparation of these so-called 'Tuberculins' or Extracts is given.

40.../

PREPARATION OF SO-CALLED 'TUBERCULINS' OR EXTRACTS FROM ACID-FAST ORGANISMS - NOT M.TUBERCULOSIS

Cultures of these organisms were made on Serum Agar slants, and within 24-36 hours, some of these had grown to such an extent, that on the water of condensation, very light pellicle material had been formed. These had been incubated at 37° C. These were:-

(a) M.Smegmatis;

(b) B.Phlei;

(c) Moellers M.Bacillus;

(d) M. of Rabinowitz.

To all tubes of cultures, a few cubic centimetres of Hartley's Broth were introduced, and after 24 hours, light pellicle material had grown.

After being incubated for 2 days at 37° C., the pellicle material had covered the entire surface of the Broth in the tubes. In all cases, with the exception of Moellers M. Bacillus, the growth quickly became thick. In the case of Moellers' M. Bacillus it quickly covered the surface but the pellicle was very thin, fragile looking and difficult to handle. Two days later, pellicle material was taken from the surface of the Broth in the tubes, and introduced on to the surface of Broth, in flasks, and this was again incubated at 37° C. In from 2-3 days, the entire surface was covered with a growth that became thicker and thicker. Moellers M. Bacillus behaved in the same way at the beginning in the flasks, as it did in the tubes.

Pellicle material in the tubes of broth. These growths were kept at room temperature but in spite of this the pellicle material became thicker. After 15 days the following was observed.

(a).../