- (a) M. Smegmatis The pellicle material has, on surface and just below it, assumed a light orange colour, and the under-surface of the pellicle material that was growing in the broth had the appearance of miniature bushy white branches on short stalks. A small amount of material has sunk to bottom.
- (b) M. Phlei The whole of the pellicle material is a marked orange colour and extends into the broth for some distance. A small amount has sunk.
- (c) M. of Moeller The pellicle material is cream in colour, and extends into the broth. Very small amounts have sunk.
- (d) M. of Rabinowitz The pellicle material is cream in colour. A small amount has sunk.

The broth in all cases is water-clear.

<u>Pellicle material in the flasks</u> - This was seeded out on 10/8/33, and the following is a description of the growths on 23/8/33 - 12 days later.

- (a) M. Smegmatis The surface of the broth is entirely covered by the seed material and it has spread a short distance up the inner surface of the flask. The growing surface is wrinkled, showing well defined large folds; it is somewhat creamy in colour and not very thick. The growth has sunk in fair quantities and the immersed growth is breaking away from the surface of the flask.
- (b) M. Phlei The surface of the broth is entirely covered by the seed material, and it has spread a short distance up the surface of the flask.

  The growing surface is irregular, and raised into very many irregular elevations; it is orange in colour and thick. Very little of the growth

- has sunk but it is starting to break away from the inner surface of the flask. The surface of the growth is somewhat dry in appearance.
- (c) M. of Moeller The surface of the broth is entirely covered by the seed material, and it has spread a short distance up the surface of the flask. The growing surface is a very fine wrinkled mass; the folds are thin and especially numerous; it is very pale brown in colour and fairly thick. A small amount of growth has sunk. The surface of the growth is fairly dry.
- (d) M. of Rabinowitz The surface of the broth is entirely covered by the seed material and it has spread a short distance up the surface of the flask. The growing surface is irregularly wrinkled, being made up of large and small folds, which are thick and fine in appearance. It is cream coloured, thin and moist in appearance. A few lumps of growth have sunk.

In the case of all of these organisms, where a second batch was seeded out on 14/8/33 with thicker pellicle material, growth by the 23/8/33 was almost as good as those seeded on 10/8/33.

## Actual Preparation of the 'Tuberculins" or Extracts.

On 1/9/33 work was started on all flasks. They had been steamed for a short while, the day previous, with the result, that all surface growth had sunk to the bottom in a thick mass and the supernatant liquid was quite clear. Filtration was carried out by means of small funnels and small filter papers. This was very slow and for each extract several filter papers had to be used. The filtrate in most cases was clear, but in some, a slight fine surface scum was noted. In these cases, another filtration was carried.../

carried out and the filtrate, this time, was quite clear. These were now left 24 hours in an ice chest to see if any further deposit would be thrown down - they remained as before. Concentration to 1-10 strength was carried out in white porcelain evaporating dishes over a bunsen flame. The remaining liquid was now once more filtered into small bottles - this filtration only proceeded slowly on account of the liquid being somewhat thick in consistency. It was perfectly clear and the 'tuberculins' varied in colour from light to dark brown.

During the filtering off, of the killed organisms, the only ones that had any odour at all, were M. Rabinowitz and M. Smegmatis preparations.

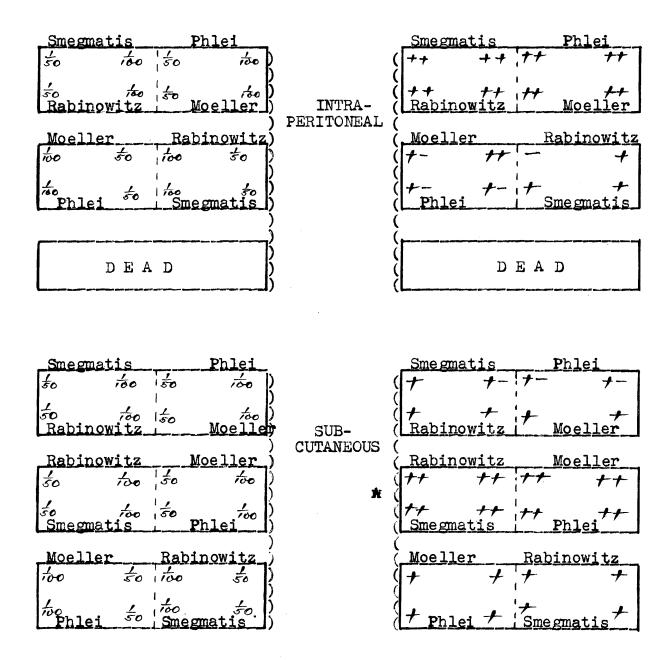
In addition to the guinea pigs inoculated with acid-fast organisms, not M. Tuberculosis, other guinea pigs were infected with -

- (a) Diphtheroids from an infected udder;
- (b) Diphtheroids from a calf;
- (c) T.100 (a human strain) of M. Tuberculosis;
- (d) Br.abortus.

Extracts prepared from the acid-fast organisms, not M. Tuberculosis, were tested on all these artificially infected guinea pigs.

# CAGE 1 - GUINEA PIGS INFECTED WITH M.PHLEI SUSPENDED IN LIQUID PARAFFIN

Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of extracts from acidfast organisms, not M. Tuberculosis.



\* 8/9/33. Reaction of M. Phlei in this guinea pig more marked.

#### RESULTS:

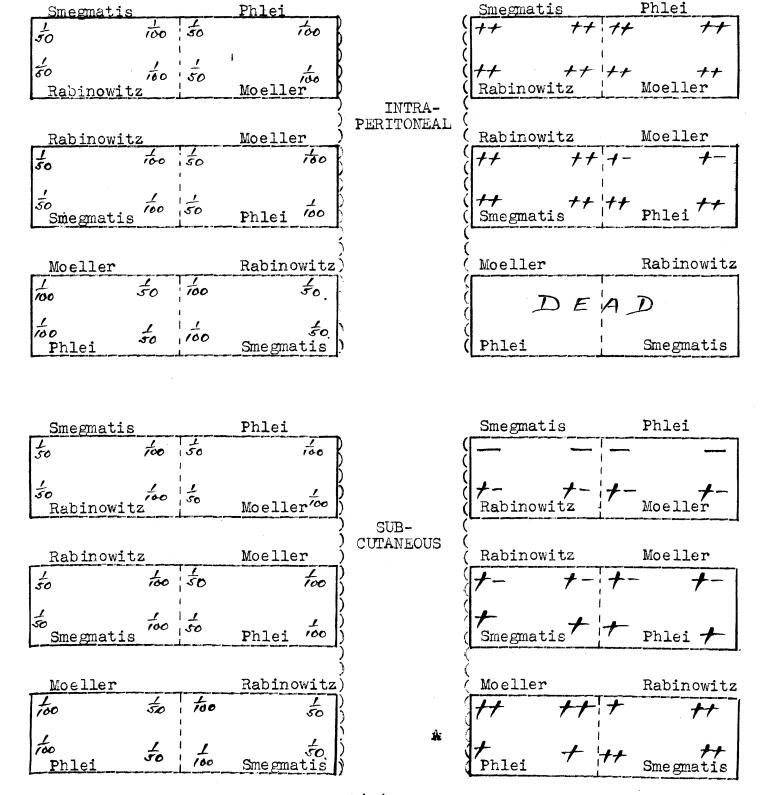
Reactions were given to all test Extracts. Controls regative.

CAGE 2 - GUINEA PIGS INFECTED WITH M.RABINOWITZ SUSPENDED IN LIQUID PARAFFIN

1 1

Tested with 50 and 100 dilutions of Extracts from

Acid-fast organisms not M.Tuberculosis.



★ PHLET reaction faded markedly 8/9/33.

RESULTS: Reactions were given to all test extracts.

In one guinea pig from the group injected subcutaneously, no reactions were given to Smegmatis and Phlei reagents, but in the other two guinea pigs the reactions were fairly marked.

## CAGE 3 - GUINEA PIGS INFECTED WITH M.SMEGMATIS SUSPENDED IN LIQUID PARAFFIN

Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of Extracts from Acid-fast organisms not M. Tuberculosis.

PERITONEAL Smegmatis SUB-CUTANEXUUS Moeller Rabinowitz

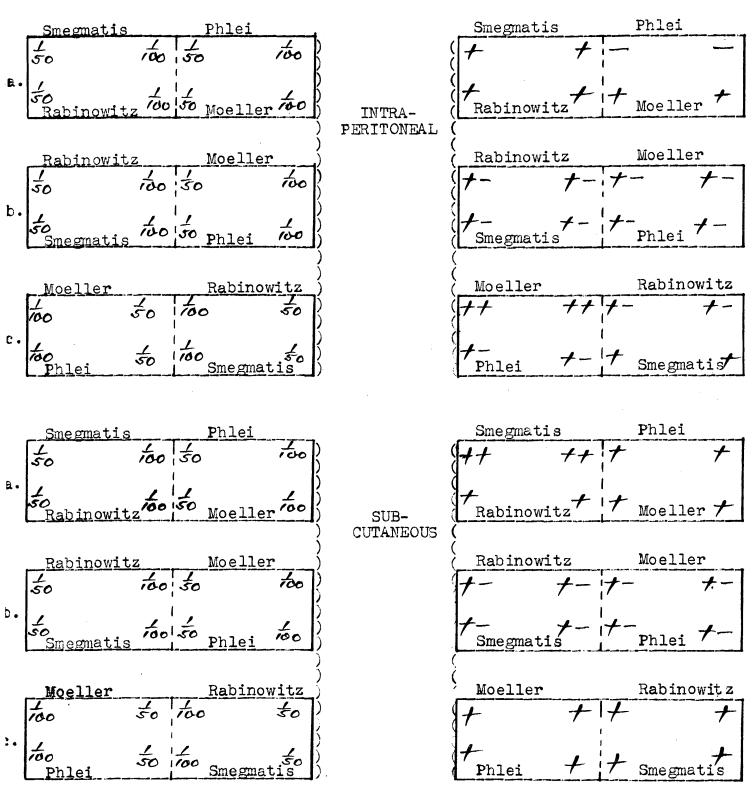
8/9/33.

RESULTS: Reactions were given to all test extracts.

47.../

CAGE 4 - GUINEA PIGS INFECTED WITH MOELLER'S MIST BACILLUS SUSPENDED IN LIQUID PARAFFIN

Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of extracts from acid-fast organisms not Bacillus Tuberculosis.



RESULTS: In the case of guinea pigs injected by the Intraperitoneal route, reactions were slight except in No. c, i.e. Moeller extract against Moeller infected guinea pigs.

In the case of guinea pigs injected by the Subcutaneous route, reactions were slight except in No. a, i.e. Smega Bacillus extract against Moeller infected guinea pigs.

To sum up reactions with all extract were slight.

CAGE 5 - GUINEA PIGS INFECTED WITH A STRAIN OF C.PREIZ-NOCARD ISOLATED FROM A HORSE Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of Extracts from

Acid-fast organisms not M. Tuberculosis.

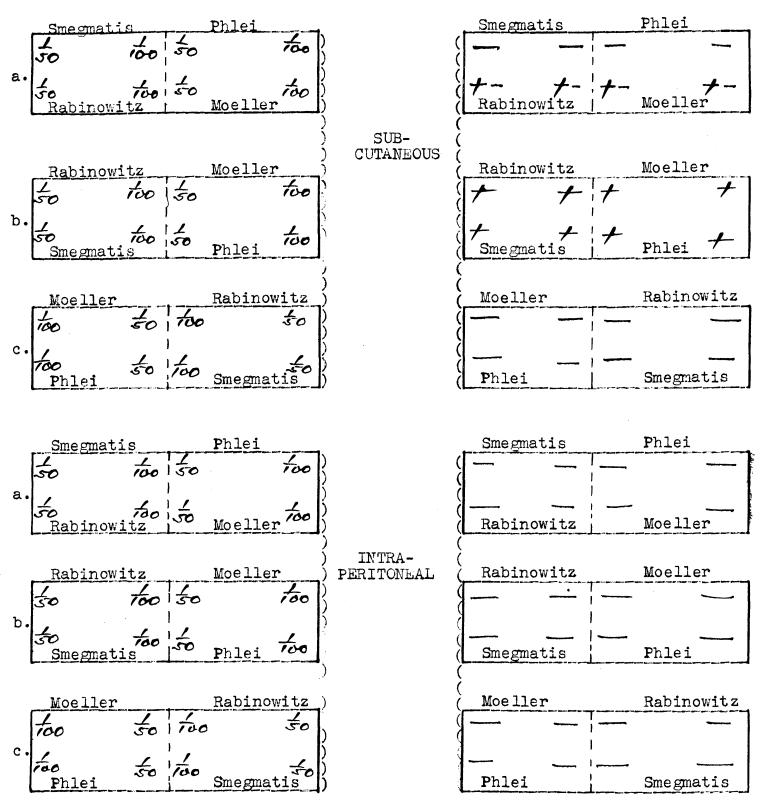
Smegmatis Phlei		Smegmatis	Phlei
50 100 50 100			
Rabinowitz 100 50 Moeller 100		( Rabinowitz	Moeller
Rabinowitz Moeller	INTRA- PERITONEAL	( ( ( Rabinowitz	Moeller
50 100 50 100			
50 smeomatis to 50 Phlei to		( Smegmatis	Phlei
Moeller Rabinowitz		( ( Moeller	Rabinowit z
too \$0 100 \$0			
too to too Smegmatis		( Phlei	Smegmatis
Smegmatis Phlei		Smegmatis	Phlei
50 100 50 100			
Rabinowitz so Moeller too		Rabinowitz	Moeller
· ·		\ <b></b>	أأحبجت مبرسي مسيني مسيمي سيومسيس ببيدسرس
Rahinowitz Moeller	SUB- CUTANEOUS	( ( Rabinowitz	Moeller
Rabinowitz Moeller		(	grand germit managaran gamangkaran dalimit karan adari karan dan magan in dalam disemb
The same of the sa		( ( Rabinowitz	grand germit managaran gamangkaran dalimit karan adari karan dan magan in dalam disemb
so so so so so so Smegmatis Phlei		Rabinowitz  DE P	D.
so   so   so   so   so   so   so   so		Rabinowitz  DE  Smegmatis	Phlei

## RESULTS:

No reactions given to any of the test extracts.

# CAGE 6 - GUINEA PIGS INFECTED WITH A STRAIN OF C.PREIZ-NOCARD ISOLATED FROM A SHEEP

Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of Extracts from Acid-fast organisms not M. Tuberculosis.



#### RESULTS:

Slight reactions were given in two guinea pigs of the group that were inoculated subcutaneously, but by the following day, these had completely faded.

## <u>DATE 7/9/33</u>. Inoculated 1/8/33.

## CAGE 7 - GUINEA PIGS INFECTED WITH C.PYOGENES

Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of Extracts from Acid-fast organisms not M. Tuberculosis.

	Smegmatis	Phlei	Smegmatis	Phlei
	50 100	50 100		
a.	rabinowitz 600	Noeller 100	Rabinowitz	Moeller
	Rabinowitz	Moeller	Rabinowitz	Moeller
	50 100	50 100	{ + + +	+
b.	Smegmatis 60	fo Phlei	Smegmatis	Phlei —
	Moeller	Rabinowitz	Moeller	Rabinowitz
	100 50	100 50	{ <del>  +-                                  </del>	<i>†</i> -
c.	//so //so	100 Smegmatis	)	≠- Smegmatis
	Smegmatis	Phlei	Smegmatis	Phlei
	50 100	र्जं रंजं	\\ \psi -	+-
a.	so /oo Raginowitz	so Moeller 100	Rabinowitz +- +-	Moeller
	Rabinowitz	Moeller	) ( } Rabinowitz	Moeller
	التراسي بالبار والمراسين والمراسية والمراسية والمراسية والمراسة والمراسية والمراسية والمراسية	50 100		
b.	/ so /oo Smegnatis	fo Phlei 100	Smegmatis	Phlei
	Moeller	Rabinowitz	Moeller	Rabinowitz
	too 50	too to		
C.	/oo /so Phlei	Smegmatis	Phlei	Smegmatis

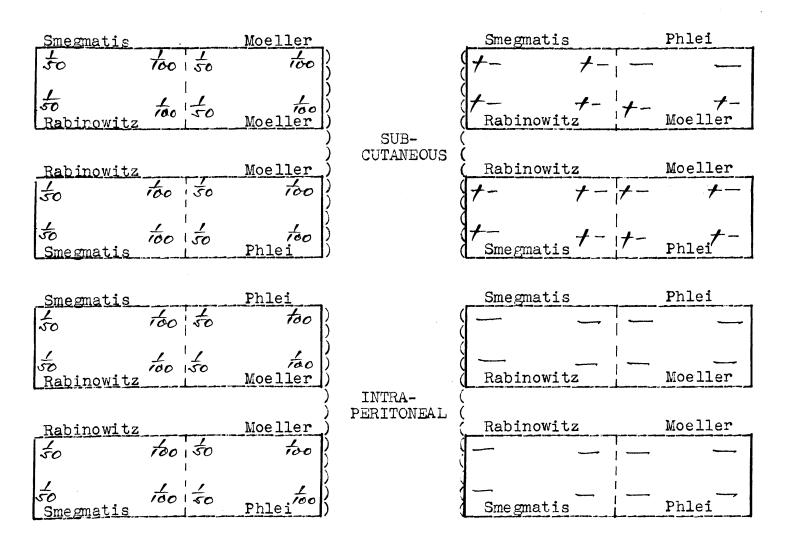
RESULTS: Slight reactions in 2 guinea pigs of intraperitoneal group, and in 1 of subcutaneous group.

# Smegmatis Phlei Smegmatis Phlei To 1/00 50 1/00 Rabinowitz 1/00 1/00 Rabinowitz 1/00 1/00 Rabinowitz 1/00 1/00

RESULTS: No reactions in controls.

CAGES I & J - GUINEA PIGS INFECTED WITH A DIPHTHEROID ISOLATED FROM AN UDDER OF A COW

Tested with  $\frac{1}{50}$  and  $\frac{1}{100}$  dilutions of Extracts from Acid-fast organisms not Bacillus Tuberculosis.

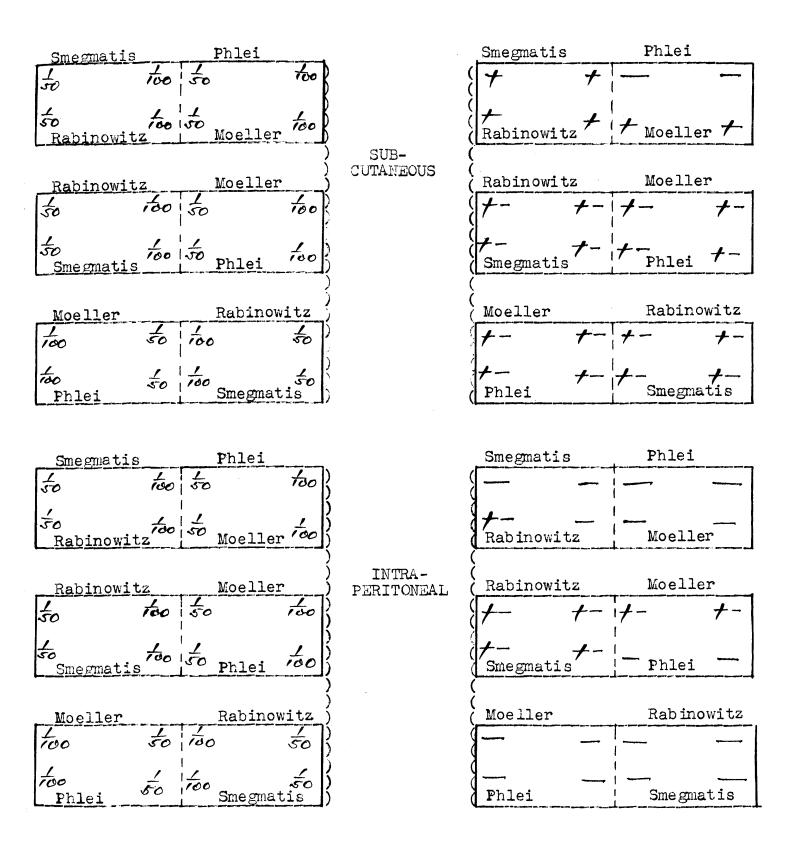


## RESULTS:

Doubtful or very slight reactions were given to test extracts in the case of guinea pigs inoculated by the Subcutaneous route.

CAGE G & H - GUINEA PIGS INFECTED WITH DIPHTHEROID ISOLATED FROM A CALF

Tested with dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Extracts from Acid-fast organisms not Bacillus Tuberculosis.



## RESULTS:

Doubtful or very slight reactions were given to test extracts in the case of guinea pigs inoculated by the Subcutaneous route.

53.../

CAGE K - GUINEA PIGS INFECTED INTRAMUSCULARLY WITH A HUMAN STRAIN OF M.TUBERCULOSIS - T.100

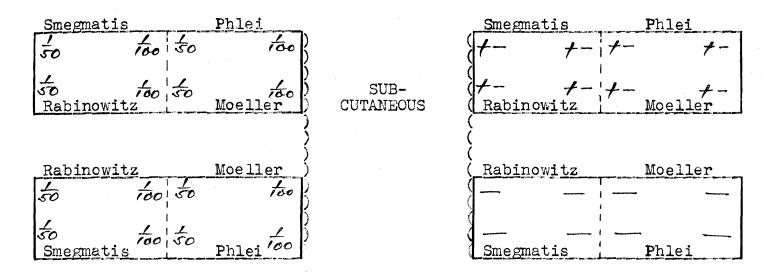
Tested with dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of extracts from Acid-fast organisms not MBacillus Tuberculosis.

Smegmatis Phlei	Smegmatis Phlei
50 too 50 too	+-+- +-
Loso too too Moeller too	Rabinowitz + H Moeller +
Rabinowitz Moeller	Rabinowitz Moeller
50 100 50 100	++++
Smegmatis 100 SO Phlei 100	t t-Phlei t-
<u>Moeller Rabinowitz</u>	Moeller Rabinowitz
100 50 100 50	+ + + +-
Loo Joo Joo Joo Phlei Smegmatis	+- +- +- +- Smegmatis
Smegmatis Phlei	Smegmatis Phlei
Smegmatis Phlei	Sine gina cis
So So Moeller'so	Rabinowitz Moeller

RESULTS: Very slight reactions to test extracts in three out of four guinea pigs.

CAGE L - GUINEA PIGS INFECTED SUBCUTANEOUSLY WITH A STRAIN OF BR.ABORTUS

Tested with  $\frac{1}{50}$  to  $\frac{1}{100}$  dilutions of Extracts from Acid-fast organisms not Bacillus Tuberculosis.



RESULTS: Doubtful reactions in the case of one guinea pig.

## SUMMARY

From this experiment it would appear as if the Extracts made from M. Phlei, the Butter bacillus of Rabinowitz, Moeller's Mist Bacillus and the Smegma Bacillus - all acid-fast organisms - have something common to all members of this group as is shown by the cross intradermal reactions. The extract of any one member, e.g. Smegma bacillus extract, is not specific for its own organism, i.e. Smegma bacillus.

No definite reactions were given with these Extracts when tested against M. Tuberculosis infected guinea pigs.

The same applies to guinea pigs infected with the Preiz-Nocard organism, Diphtheroid organisms or Br.abortus.

The extracts are evidently specific only for members of their own group of organisms and for no others.

It was decided to repeat the former experiment using dilutions of the Extracts made from this group of acid-fast organisms not M. Tuberculosis, and similar dilutions of Tuberculin.

More guinea pigs were now taken and inoculated in the same manner with acid-fast organisms (not M.Tuberculosis).

DATE OF INOCULATION 30/10/33.

		Channel of Arti-		
A. :		Subcutaneous Intraperitoneal Controls	M.Phlei + Liquid Paraffin M.Phlei + Liquid Paraffin Controls	1 c.c. 1 c.c.
В.	3 3 3	Subcutaneous Intraperitoneal Controls	M.Rabinowitz + Liquid Paraffin M.Rabinowitz + Liquid Paraffin Controls	1 c.c. 1 c.c.
C. :	3 3 3	Subcutaneous Intraperitoneal Controls	M.Smegmatis + Liquid Paraffin M.Smegmatis + Liquid Paraffin Controls	l c.c. l c.c.
D. :	3 3 <b>3</b>	Subcutaneous Intraperitoneal Controls	B. of Moeller (Mist) * Liquid Paraffin B. of Moeller (Mist) * Liquid Paraffin Controls	
E. :	3 3 3	Subcutaneous Intraperitoneal Controls	M.Phlei from lesions & Liquid Paraffin M.Phlei from lesions & Liquid Paraffin Controls	l c.c. l c.c.
F. :	3 3	Subcutaneous	M.Rabinowitz from lesions * Liquid Paraffin M.Rabinowitz from lesions * Liquid	1 c.c.
	3 :	Intaperitoneal Controls	Paraffin Controls	1.c.c.

Controls were placed in the same cages as their respective groups of inoculated guinea pigs.

# CAGE A - GUINEA PIGS INFECTED WITH M.PHLEI SUSPENDED IN LIQUID PARAFFIN

Tested with  $\frac{1}{50}$  dilutions of Extracts from Acid-fast organisms not M. Tuberculosis and dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Tuberculin.

Smegma	Phlei	Rabino	Moelle	r	Smegma	Phlei	Rabino	Moeller
50	50	50	50	) (	+	+	+	+
√ 50 T.B.	100 T.B.	والمراقبة		) ( ) ( ) INTRA- ( ) PERI- (	T.B.	7.B.	-man Whitehald are required to the partition	- Change Strongers relative game again (SSS), game
Moeller	Phlei	Rabino	Smegma	TONEAL (	Moeller	Phlei	Rabino	Smegma
50	50	50	50	) )	+-	+	+-	+-
50 T.B.	7.B.	astropasti kanan santi kitik kanan kilana pakati kanan		) ( ) (	7.B.	7- T.B.		
Phlei_	T.B.	T.B.	Smegma	) () ()	Phlei	Т.В.	T.B.	Smegma
50	50	100	50	) (	+-	+	+-	+
50 Rabino	خ Moeller			) ( ) (	≠ Rabino	/ Moel		
Smegma	Phlei	Rabino	Moeller		Smegma	Phlei	Rabino	Moeller
50	50	50	50	) (		T - T	ריי תו	
50	100			) (	m T)	D I T.B.	עו פ	
T.B.	T.B.	and the latest description of the latest des		SUB- (	T.B.	1 • D •		
Moeller		Rabino	Smegma	TANEOUS (	Moeller	Phlei	Rabino	Smegma
50	50	50	50	)		Anniphyddiniau di	-	-
₹.B.	/00 T.B.	nampanak pamili pam		) (	 T.B.	T.B.	paraganta annativo galetagas, pa que un	
T.B.	T.B.	Moeller	Smegma	) ) - ( )	T.B.	T.B.	Moeller	Smegma
50	100	50	50	<b>)</b> (		<del>-</del>	<del>-</del>	+-
50 Phlei	خ ر Rabir	no		) ) (	/- Phlei	/- Rabin	0	

RESULTS: Reactions were obtained with all extracts but only in those guinea pigs that were injected intraperitoneally.

Reactions were also given to the Tuberculin (Standard) in three guinea pigs.

CAGE B - GUINEA PIGS INFECTED WITH M.RABINOWITZ SUSPENDED IN LIQUID PARAFFIN

Tested with dilutions of  $\frac{1}{50}$  of Extract from Acid-fast organisms not M. Tuberculosis and dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Tuberculin.

megma	Phlei	Rabino	Moeller		Smegma	Phlei	Rabino	Moeller
50	50	50	50	)	<u> </u>			
1				) )				
50 B.	100			) : TNUID A	( T.B.	T.B.		
LaBa	T.B.			INTRA- PERI-	( <u>1.D.</u>	I • D •	وري والمستوني ويستوني ويست والمستون	
3.7	ma a e	T) - 1 1		TONEAL.	(Man 2 lam	Phlei	Rabino	Smegma
	Phlei	Rabino /	Smegma /	<b>)</b>	Moeller	FIITET	RAULIIO	Smegma 7
50	50	50	50	)			-	<b>*********</b>
1	too			) :	$\{ - \}$	Magazina		
50 L.B.	T.B.			)	T.B.	T.B.	التحديد التحديد الأستين المعدد	
				<b>)</b>	(			
Phlei	T.B.	T.B.	Rabino	<i>)</i>	Phlei	T.B.	T.B.	Rabino
50	50	100	50		9—	+-	<del>/-</del>	
				<i>)</i> )				
₹0 Voeller	_	€o megma					m o cran o	
Moeller		megma		)	Moeller	<u> </u>	megna	
Smegma	Moeller	Rabino	Phlei		Smegma	Moeller	Rabino	Phlei
50	50	50	50		1-	+-	+	
1				) )	<b>y</b> '	,	,	
50	,, T.E	0		SUB-		T.		
r.B.	T•E	•	}	CU- TANEOUS	T.B.	I •	D•	
				<b>)</b>		****	Dalana	<b>O</b>
	Phlei ,	Rabino	,	) <b>)</b>	Moeller	Phlei	Rabino	
50	50	50	50	)	1		+	++
50	<u>ئ</u> ے	50			1_		-	
T.B.		В.	ß	, )	T.B.		T.B.	

## RESULTS:

Very slight reactions were given, probably due to the guinea pigs not being properly sensitised.

# CAGE C - GUINEA PIGS INFECTED WITH SMEGMATIS SUSPENDED IN LIQUID PARAFFIN

Tested with dilutions of  $\frac{1}{50}$  of Extracts from Acid-fast organisms not M. Tuberculosis and dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Tuberculin.

Smegma	Moeller	Phlei	Rabino		Smegma	Moeller	Phlei	Rabino
50	50	±0	50		1+	+	+	++
ļ			5					
70 T.B.	ive T.B		R	INTRA-	( T.B.	T.B.		
200 mm	A STATE OF THE PERSON NAMED OF THE PERSON NAME	Barrer (MARIE) de le mandérie de l'Alle de l'A	)	PERI-		C almost apartie die Commission democratica de les especiales de la commission de la commis	ti <del>gerri ga a Kalenda gipa Tibadad gaddi <b>taman</b>g add</del>	i est nassastatu fili dagun vijamunu galannakanyun delimmi dagunda
Т.В.	T.B	Smegma	Moeller)	TONEAL	(T.B.	T.B.	Sme gma	Moeller
50	100	50	50		1	+-	++	++
į.			])		1	r		
50 Phlei	Rabin		Į)		1+	++		
Phlei	<u> Rabin</u>				Phlei	Rabin	. <u>O</u>	where the section desired species are the section of the section o
	•							
<b>L</b>			70 - 1		Cro o cros	Phlei	Moeller	Rabi no
Smegma		Moeller	Rabino (		Smegma /	+ IIILEI	Moe Tiel.	- Nabi no
50	50	50	to		<b>d</b> '	/	/	
50	100		В		1—	<b>Constitution</b>		
F.B.	T.B.			SUB-	<u>r.B.</u>	T.B.	المعادد	
			)	CU- TANEOUS	(			
r.B.	T.B.	Smegma	Phlei (		T.B.	T.B.	Sme gma	Phlei
50	too	50	50		<b>\{</b>	-	++	++
1,	<b>1</b>		ß					
50	50.		D			++		
Moeller	Rabin	O	13		Moeller	Rabin	.0	1

### RESULTS:

Reactions throughout but none with Tuberculin.

CAGE D - GUINEA PIGS INFECTED WITH THE MIST BACILLUS OF MOELLER SUSPENDED IN LIQUID PARAFFIN

Tested with dilutions of  $\frac{1}{50}$  of Extracts from Acid-fast organisms not M. Tuberculosis and dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Tuberculin.

Smegma

Moeller

Phlei Rabino

50	50	50	50	)	1	+-	+-	+-
50 T.B.	to T.B			) ) ! INTRA-	(T.B.	T.B	•	
T.B.	T.B.	Smegma	Moeller	PERI- TONEAL	(T.B.	Т.В.	Sme gma	Moeller
50	100		50	) )	Phlei	Rabino		
<u>Phlei</u> Rabino	Rabi Phlei	· · · · · · · · · · · · · · · · · · ·	· Sme gma	) ) )	(Rabino	Phlei	Moeller	Smegma
50	50	50	50		1	+	+	+
50 T.B.	7.00 T.B.	بالمستعدد والمستعدد		) )	T.B.	T.B.	materians manifests michigan i dell'agent bui	
Smegma	Moeller	Phlei	Rabir	10	Smegma	Moeller	Phlei	Rabi no
50	50	50	50	SUB- CU- TANEOUS	1+	++	++	++

## RESULTS:

Moeller Phlei Rabino

Slight reactions in the guinea pigs inoculated by the intraperitoneal route, but very marked in the guinea pigs inoculated by the subcutaneous route. No reactions were given to Tuberculin.

Rabino

50

CAGE E - GUINEA PIGS INCCULATED WITH M.PHLEI SUSPENDED IN LIQUID PARAFFIN

(This strain of M. Phlei was isolated from lesions in a guinea pig infected artificially with M.Phlei).

Tested with dilutions of  $\frac{1}{50}$  of Tuberculins from Acid-fast organisms not M. Tuberculosis and dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Tuberculin.

INTRA-( ++

/ 50 T.B.	70 e T.B.			PERI- TONEAL	( <del>/</del> T.B.	<b>≠</b> T.B.		
Smeema	Moeller	Phlei 50	Rabino	)	Smegma	Moeller ++	Phlei	Rabino ++
50 T.B.	/00 T.B.	,		) ) SUB- ) CU- ) TANEOUS	(T.B.	<b>T.</b> E	- 3.	
T.B.  50  50	T.B.	Smegma 50	Moeller 5-0	TANEOUS	(T.B.	T.B	Smegma /	Moeller /
Phlei Rabino		Moeller	Smegma)		(Phlei ( (Rabino	Rabi Phlei		Smegma
50 50 T.B.	to T.B.	50 	50	) ) )	(T.B.	7.E	<i>+</i>	++

#### RESULTS:

Moeller

50

Good reactions with all test extracts were obtained. As in the guinea pigs infected with M. Phlei from Cage A of this experiment, reactions although not marked, were given to Tuberculin.

CAGE F - GUINEA PIGS INFECTED WITH M.RABINOWITZ SUSPENDED IN LIQUID PARAFFIN

This strain of the Bacillus was obtained from lesions in a guinea pig infected artificially with M.Rabinowitz.

Tested with dilutions of  $\frac{1}{50}$  of Extracts from Acid-fast organisms not M. Tuberculosis and dilutions of  $\frac{1}{50}$  and  $\frac{1}{100}$  of Tuberculin.

Smegma	Moeller	Rabino	Phlei		Smegma	Moeller	Rabino	Phlei
50	<del>š</del> o	50	50		+	+	+	+
T.B.	/3-0 T.B.				<b>/</b> - Т.В.	f.1	В.	
				) INTRA- ( ) PERI- (	(			
T.B.	T.B.	Smegma	Moeller	TONEAL	T.B.	T.B.	Smegma	Moeller
50	roo	50	50	) ì		-	++	+
so Rabino	√ √ Phle	i		) )	/ Rabino	Ph	lei	
				)	( (	<b>35.1.7</b> 1\$	N/a - 7 7 a	Cm a cms
Rabino	Phlei	Moeller /	Smegma 50	) }	Rabino	Phlei	Moeller	Smegma
50	50	50	50	<b>)</b>				
50 T.B.	750 T.B.	) 		)	Т.В.	T.	B.	
Smegma L So L So T.B.	Moeller 50 70 T.B.	Rabino	Phlei	) ) ) SUB- ) CU-	Smegma  T.B.	Moeller — T.	- Angelenned	Phlei
T.B.	Т.В.	Smegma	Moeller	TANEOUS	( ( T.B.	T.B.	Smegma	Moeller
50	100	50	50				+	+
50 Rabino	Phle	) 2 <b>i</b>		)	Rabino	Ph	lei	
Rabino	Phlei	Moeller	Smegma		( Rabino	Phlei	Moeller	Smegma
50	50	50	50	)	1	+	+	+
₹0 T.B.	700 T.B.	·		)	T.B.	T.	В.	
	RESULTS:	Re	actions v	were not n	narked but	t no reac	tions wer	re.

Reactions were not marked but no reactions were given to Tuberculin.

## CONTROL

Smegma	Moeller	Rabino	Phlei	Smegma	Moeller	Rabino	Phlei
50	50	50	50		-	***************************************	
50 T.B.	T.B	0,		T.B.	T.B		
RES	ULTS: Neg	ative.				62/	

#### SUMMARY:

These results confirmed those obtained in the previous experiment. No reactions were given to Tuberculin except slight ones in the case of guinea pigs infected with M. Phlei.

In order to end up this work it was decided to utilize the guinea pigs that had recovered from the test of 30/10/33. These were again tested with the extracts prepared from the group of acid-fast organisms used throughout this work, and a preparation called Anaexo-Phlein prepared from the M. Phlei. This preparation was made on the same lines as Anaexo-Tuberculin as described by Garet and Zeitoun (1933) Rec. de Med. Vet de l'Ecole Alfort.

109 - 9 - 513-18. L'anaexotuberculine.

The preparation is as follows:-

A human or bovine strain of Bacillus Tuberculosis is grown on 5% glycerine peptone broth for 4 to 6 weeks. The broth of the culture below the scum, formed by the growth, is collected by very slow aspiration, by introducing a pipette very carefully below the pellicle growth of the bacilli. The liquid obtained should be clear and free from fragments of pellicle, which should be left intact. Filter this clear liquid and add .5% Formalin. Dose for cattle .25 cubic centimetres for intradermal test.

This was reviewed in the Veterinary Record No. 48 Volume XIII of December 2nd, 1933.

CAGE A - GUINEA PIGS INOCULATED WITH M. PHLEI SUSPENDED IN LIQUID PARAFFIN

Tested with  $\frac{1}{50}$  dilutions of Extracts from acid-fast organisms not M. Tuberculosis and Anaexo Phlein.

Smegma	Phlei	Rabino	<u> Moelle</u> r	•	Sme gma	Phlei	Rabino	Moeller
50	50	50	50	) )	{ ++	++	++	++
to.				)	(Phlei (1	L)		
Phlei (	1)	د همی کاری کی این این این کاری کاری کاری کاری کاری کاری کاری کاری		INTRA-	/ / (ce	ent.necr.	<u>)</u>	B ; :: 78
_				TONEAL	(	**1 7	Dala da a	<b>G</b>
Moeller 50	Phlei	Rabino	Smegma	) )	(Moeller	Phlei  //	Rabino ++	Smegma ++
50	50	50	50	)	(Phlei (I		, ,	
50	- \				++	- /		
Phlei (	1)	ر والديد النبيع الدينانية من الاستوادات الاستوادات الاستوادات		)	(	adi seallusidh; cub milli milli milli milli suid aidd	inimit reed vertice, all while state and produces	
Phlei 1	Phlei(l)	Smegma	Rabino		Phlei	Phlei(1)	Smegma	Rabino
50	50	50	50	· )	-	-		p
150					Moeller			
Moeller				SUB-			North Tolks The Special Property and Special Property Company	
				CU- TANEOUS	(			
Smegma_	Phlei	Rabino	<u>Moeller</u>	)	Smegma	Phlei	Rabino	Moeller
50	50	50	50	) )	++	++	++	**
50.			R		Phlei (1	L)		
Phlei (1	)		K		1++	(Cent.ne	cr.)	

The site of injection of Anaexo-Phlein is marked Phlei (1).

## RESULTS:

Marked reactions with all test extracts and especially with Anaexo-Phlein, which showed in two of the guinea pigs, a central necrosis of the skin.

DATE 4/1/34 - Inoculated 30/10/33.

CAGE B - GUINEA PIGS INOCULATED WITH M. RABINOWITZ SUSPENDED IN LIQUID PARAFFIN

Tested with  $\frac{1}{50}$  dilutions of Extracts from acid-fast organisms not M. Tuberculosis and Anaexo-Phlein.

Smegma	Phlei	Rabino	Moeller		Smegma	Phlei	Rabino	Moeller
50	50	50	50		1/-	+-	+-	+-
1,	_		ß		(Phlei (1	)		
50	,		<u> </u>	INTRA-	1+-			
Phlei (]			<sup>/</sup>	PERI- TONEAL	<u>}</u>	<del></del>	ده العب، ۱۳۵۰ شمیدگذی جانبونی، مورد و	
			ý	1.01/13/12		<b>~17</b> .	Dahina	O
Moeller	Phlei	Rabino	Smegma)		Moeller	Phlei	Rabino	Sme gma
50	50	50	50		7-	+-	+-	+-
50			Į)		(Phlei (1	.)		
Phlei (]	)		K		1 +-			
	Talentee with water and water water water water water water with the state of the s	The section of the se	/		\		Australia esta esta esta esta esta esta esta est	
Dia Tarak	nu 1 . 4 / 7 \	Rabino	Moeller		Phlei P	hlei(1)	Rabino	Moeller
Phlei	Phlei(1)		·		7-	<del>/</del> -	+-	MOCTICI
50	50	50	50		(1 '		,	7-
50			R	SUB-	(Smegma			
Smegma				CU-	1			
			)	TANEOUS	(			
Moeller	Phlei	Rabino	Smegma)		(Moeller	Phlei	Rabino	Smegma
50	±0	±0	\$0				*	
50	50	50	50		(Phlei (1	)		
to			K		A 11767 (1	- /		
Phlei (	1)		5					

## RESULTS:

No reactions marked. Results inconclusive.

# CAGE C - GUINEA PIGS INOCULATED WITH M.SMEGMATIS SUSPENDED IN LIQUID PARAFFIN

Tested with dilutions  $\frac{1}{50}$  of Extracts of Acid-fast organisms not M.Tuberculosis and Anaexo-Phlein.

Smegma	Phlei	Rabino	Moeller		Smegma	Phlei	Rabino	Moeller
50	50	50	50	) \	{ +	+	+	+
				Ó	(Phlei (1	.)		ŕ
50 Phlei (	7 )			) INTRA- ) PERI-	{ } +			
THE L	±/	and a second residence of the second	······································	TONEAL		all agree a regulately resident resident resident	والمراجعة المراجعية المراجعة المراجعة الموسولية والموسولية والموسو	
Moeller	Phlei	Rabino	Smegma)	)	(Moeller	Phlei	Rabino	Smegma
50	50	<u> </u>	50	, )	1	+	+	+
	50	\$0	~0	) )	(Phlei (1	•	,	ĺ
50	- \			Ó	1	•		
Phlei (	1)	المراهد فادر فارواليونون فالتحالية فاستحد		)	(L		بدوت کی ک ۱۰ اکتاب یہ کید میں میںوں	
Smegma	Phlei	Rabino	Moeller		Sme gma	Phlei	Rabino	Moeller
50								
1 570	50	50	50		/	+	+-	+
1	50	50	50	· }	(Phlei (1	•	+-	
50		50	50 B		(Phlei (1	•	+-	
1		±0	<del>50</del>	SUB-	{ +	•	1-	
50 Phlei (	1)				{	.)	an a survivato surti setti setti surti surti surti surti setti surti surti setti surti setti surti setti surti	+
Phlei (	l) Phlei(	(1) <u>Smeg</u> m	a Phlei)	CU-	(Phlei (1	.)	an a survivato surti setti setti surti surti surti surti setti surti surti setti surti setti surti setti surti	+
50 Phlei (	1)			CU-	(Moeller	.)	an a survivato surti setti setti surti surti surti surti setti surti surti setti surti setti surti setti surti	+
Phlei (	l) Phlei(	(1) <u>Smeg</u> m	a Phlei)	CU-	{	.)	an a survivato surti setti setti surti surti surti surti setti surti surti setti surti setti surti setti surti	+

## RESULTS:

Slight reactions with all test reagents.

# CAGE D - GUINEA PIGS INOCULATED WITH MOELLER'S MIST BACILLUS SUSPENDED IN LIQUID PARAFFIN

Sme gma	Phlei	Rabino	Phlei(1)	1.	Smegma	Phlei	Rabino	Phlei(1)
50	50	50	50	)	9 —		Nagaran Carrelling	-
1				5	Moeller			
50 Moeller				) )	} —			
100-110-1	ندمة التحكمينية طامية الأداب الجوي ويبني والمهور	وحفقها مقاومها فالمهاوي فيمهون والموافق فسيقهو يشو مستهد	ر رود المدين المعيون بينيا و المدين المدين المدين المدينة المدينة المدينة المدينة المدينة المدينة المدينة المد ا	<u> </u>	(		aging, little girtle grantene gerringgen grantene	a kinnel sån til Mille den vilanden i Kilgarin i Berlin sån i i 1999 Mille dette
Moeller	Rabino	Phlei(1)	Smegma	) INTRA- (	( Moeller	Rabino	Phlei(1)	Smegma
50	50	50	50	) TONEAL			Management	
				)	Phlei			
₹o Phlei				)	<i>d</i> —			
FIITGE	re glemma. Ngghill dahilik dan ar agurill para-17 gayrin aguril			Š	` <u> </u>			maran garan garan ga wasan ga
Smegma	Phlei	Rabino	Moeller	)	( ( <b>P</b> hlei(1)	Smegma	Rabino	Moeller
\$0	1 50	£ 50	50					
		0.2		)	Phlei			
ر خo Phlei					<b>_</b>			
FITCI	The state of the	entingentiaritm eticili pietit gayay gyvre eticrifassos t	المتحربين للأحمل سندية الأحل المتحربين وسيد	,	·		رسين جيورانده السائلانية المتواقعة المن	المراجع والمحاولة المراجع والمجاور المحاولة والمحاولة والمحاولة المحاولة والمحاولة وال
Sme gma	Phlei	Rabino	Moeller					
50	50	£ 50	<u>/</u> 50	CITO		g mark garantintanum gipunin gun historian gunum digunum g	Band Carrie (Stud State) Carrie (Stude Assertingues (S	
				SUB- CU-		DEA	D	
≠ 50. Phlei (1	)			TANEOUS				
					L			

## RESULTS:

Negative.

## <u>DATE 4/1/34</u> - Inoculated 30/10/33.

<u>Phlei Rabino Moeller</u>

Smegma

CAGE E - GUINEA PIGS INOCULATED WITH M.PHLEI SUSPENDED IN LIQUID PARAFFIN

This strain of M. Phlei was obtained from a guinea pig artificially infected with M. Phlei.

Tested with dilutions  $\frac{1}{50}$  of Extracts of Acid-fast organisms not M. Tuberculosis and Anaexo-Phlein.

Smegma

Phlei

Rabino

Moeller

50 50	<u> 5</u> 0	50	20	INTRA- PERI- TONEAL	Phlei (		+	+
Phlei (	1)					(Cent.nec	rosis)	
Smegma L 50	Phlei 50	Rabino Z	Moeller 4	)	Smegma  (Phlei (	Phlei	Rabino	Moeller
%o Phlei (	1) Phlei(1)	Dohina	C'm a crea	) ) ) SUB- ) CU- )TANEOUS	(Phlei	Phlei(l)	 Rabino	Smegma
Phlei :  50  Moeller	1 50	Rabino \$0	Smegma <u>'</u>	) ) ) ) ) )	(Moeller		1001110	<b>D</b> inegula
Rabino  L So. Phlei (	Moeller	Phlei L So	Smegma	) ) ) ) )	(Rabino (Phlei (	Moeller +- 1)	Phlei	Smegma +-

#### RESULTS:

Reactions were obtained with all test extracts used. Marked reactions were seen with the Anaexo-Phlein used on guinea pigs inoculated with M. Phlei by the intraperitoneal route.

## <u>DATE 4/1/34</u> - Inoculated 30/10/33.

CAGE F - GUINEA PIGS INOCULATED WITH M.RABINOWITZ SUSPENDED IN LIQUID PARAFFIN

This strain was obtained from lesions in a guinea pig artificially infected with M. Rabinowitz.

Tested with  $\frac{1}{50}$  dilutions of Extracts of Acid-fast organisms not M. Tuberculosis and Anaexo-Phlein.

Smegma	Phlei	Rabino	Moeller		Smegma	Phlei	Rabino	Moeller
50	50	50	50	)	9 —			•
150					Phlei(1	)		
Phlei (	1)			) INTRA- ) PERI-	<u> </u>			
		~		TONEAL	(Dla Jari	Dobino	Smegma	Phlei(1)
Phlei	Rabino	Smegma /	Phlei(1)	<b>)</b>	(Phlei	Rabino	# -	111161 (1)
50	50	50	50		Moeller			
50 Moeller				) )	{ +			
					(			
Rabino	Smegma	Phlei	Moeller	) )	Rabino	Smegma	Phlei	Moeller
50	50	50	50	)	{	· +	-	+
50				)	Phlei (	1)		
Phlei (	1)	مواولی میدو مددو ««دوروس» («دوروس» و «دوروس» که		)	(		المال المالية بالمراجعة فالمالة ومين الفاعرات بين	
Smegma	Phlei	Rabino	Moeller		Sme gma	Phlei	Rabino	Moeller
50	50	50	50	)	1	7-	+	+
to				) )	Phlei (	1)	·	, 
Phlei (	1)			SUB- CU-				
				TANEOUS	(			
Phlei	Rabino /	Moeller	Smegma	) )	<u>{</u>	<del></del>		
50	50	50	50		Į (	DI	E D	
5€0 Phlei (	1)			) )	al and a second			
ىلىدە دەمى <del>كالىكىلىنىڭ دالاسىل</del>				•	<u> </u>			

RESULTS: Slight reactions with all test reagents.

## CONTROL

Phlei(l)	Moeller	Smegma	Rabino	Phlei(1)	<u>Moeller</u>	Sme gma	Rabino
y yo y So Phlei	20 1	±0	50	Phlei			

RESULTS: Negative.

SUMWARY: This experiment also confirms work done in the two previous experiments. The reactions obtained by the use of so-called 'Anaexo-Phlein' seems to indicate that this testing agent contains something that is specific for its own organism as judged by the reaction given in guinea pigs artificially infected with M. Phlei.

#### **CONCLUSIONS:**

- 1. A sufficient degree of infection with the organism of Tuberculosis can be set up, by artifical means, to give reactions with Tuberculin within from 24 to 30 days. This means, that the period of 6 8 weeks usually undergone by subjects, injected with suspicious Tubercular material, can be shortened by testing the subject and a control, in from 24 to 30 days, thus expediting results of tests.
- 2. Animals infected with Br.abortus may give strongly suspicious Tubercular reactions with Tuberculin.
- 3. Reactions strongly suspicious of Positive Tuberculous reactions have been observed in cattle that were pregnant and close to the time of parturition.

  A similar case was observed in a control guinea pig.
- 4. Guinea pigs infected with Acid-fast organisms which were not strains of M.Tuberculosis did not give reactions when tested with Tuberculin by the Intradermal method.
- 5. Extracts prepared from Acid-fast organisms, not belonging to the M.Tuberculosis group, in the same way as Standard Tuberculin is prepared, appear to contain something common to all members of this group that gives reactions when tested against small animals artificially infected with separate member of this group.
- 6. Extracts prepared on the lines of L'anaexo-Tuberculin' may contain products that are specific for each member of the Acid-fast group of organisms.