VITAMIN A DEFICIENCY AND RESISTANCE AGAINST A SPECIFIC INFECTION.

PRELIMINARY REPORT.

By H. C. A. LASSEN.

From the University Hygienic Institute (Chief: Prof. L. Fridericia, M.D.) and the State Serum Institute (Chief: Th. Madsen, M.D.), Copenhagen.

Soon after experimental vitamin research began, a certain connection between vitamin A deficiency and spontaneous infections was observed in that numerous infectious foci could be demonstrated in experimental animals which had died from vitamin A deficiency. Moreover, the course of experimental infection and of reaction to injected bacterial toxins in animals on A-deficiency diet has been the subject of several investigations.

Werkman (1923, p. 247) found that intravenous injection of killed typhoid bacilli gave the same agglutinin production in rats on vitamin A-free diet as in rats on normal diet; nor could there be demonstrated any difference in the production of precipitin, haemolysin and bacteriolysin.

Werkman (1923, p. 255) also reported that rats on vitamin A-free diet showed a lowered resistance against subcutaneous injection of typhoid bacilli and intraperitoneal injection of anthrax bacilli. Finally, the same author (1923, p. 263) showed that in rats and rabbits on vitamin A-deficient diet the opsonic indexes for the typhoid bacillus and staphylococcus albus were not lowered to any extent worth mentioning.

Pritchett (Webster and Pritchett, 1924) showed that while the mortality in mice on ordinary laboratory diet after oral administration of mouse-typhoid bacilli was 80 per cent., it went right down to 10 per cent. when the mice were put on a perfectly adequate diet. In an experimental series on resistance against diphtheria toxin, Pritchett (Werkmann, Baldwin and Nelson, 1924) found the lethal dose for rats on diet deficient in vitamin A or B to be about one-half of the lethal dose for rats on normal diet.

Cramer and Kingsbury (1924) have also studied the production of typhoid agglutinin in rats on vitamin A-free diet and found it to be normal.

Pritchett (1927) has shown that the constituent in synthetic normal diet which makes this superior to ordinary laboratory diet (wheaten bread and pasteurised milk) is butter-fat. Therefore it is reasonable to assume that the increased resistance is due to the optimal content of vitamin A in the synthetic diet.

After oral administration of large doses of bacteria belonging to the paratyhoid group, Verder (1928) found no substantial difference in the degree of infection in rats on vitamin A-free diet and rats on normal diet.

In the clinic, finally, Bloch (1917) has shown that xerophthalmia in infants is due to vitamin A deficiency of the food. Bloch says: "Common to these patients was further their marked susceptibility and low resistance to secondary

infections" (conjunctivitis, chronic rhinitis, otitis, bronchitis, bronchopneumonia, dermatitis, and pyuria).

Lately, Green and Mellanby (1928) have thoroughly described the spontaneous infections in rats on vitamin A-deficient diet. These authors employ a diet, the ingredients of which, with one exception, are certainly all free of vitamin A; only the source of vitamin C—lemon juice—contains any, but very small, amount of vitamin A. So it is found, too, that the average lifetime of Green and Mellanby's animals on this diet is somewhat longer than the average lifetime of rats on the vitamin A-free diet later described (employed in the University Hygienic Institute). For the latter diet does not include any vitamin C-containing substance, as rats and mice do not require vitamin C for the maintenance of normal growth and weight.

Green and Mellanby diagnose infection of an organ only on the macroscopic evidence, and bacteriological examination is made only in a few cases. In 45 rats that died from vitamin A deficiency, only 1 was found in which no infectious focus could be demonstrated, whereas not one single instance of pronounced infectious changes was found in 50 rats on the same diet plus vitamin A. In the rats on vitamin A-deficient diet the most frequent infections were: abscess at the base of the tongue (*ca.* 87 per cent.), xerophthalmia (*ca.* 53 per cent.), infection of the urinary tract (almost 50 per cent.). Infection of the lungs, on the other hand, occurred in less than 10 per cent. From this, it is evident that it is a rule almost without exception, that rats which die from vitamin A deficiency show more or less extensive spontaneous infections, which probably are contributory causes of death, whereas spontaneous infection practically never occurs in rats which have lived a long time on adequate synthetic diet.

The work reported in the following pages tends to show that the greater susceptibility to spontaneous infection in rats on vitamin A-free diet also applies to a specific experimental infection.

At the State Serum Institute in later years, the different paratyphoid infections, after ingestion of bacterial cultures, have been the subject of a series of investigations by Ørskov, Jensen and Kobayashi (1928), Ørskov and Schmidt (1928) and Ørskov and Moltke (1928). These authors have worked with mice as experimental animals, and their results—on the basis of their special autopsy technique—have shown an extraordinary regularity in the course of the infections. This autopsy technique reveals fairly advanced deviations from normal conditions in mice.

As mice, however, lend themselves but poorly to vitamin experiments, rats have been employed. The experimental rats are bred from rats which have long been kept exclusively on a fixed, in all respects adequate, synthetic powdered food for breeding animals, in order to make sure that the young ones are as far as possible all alike with regard to vitamin depots. When the young rats are 1 month old, they are taken from the mother and entered in the experiment.

To promote a definite positive result of the experiment, it was important

to find a micro-organism and a dosage which would give a fairly strong, rather protracted, but non-fatal infection in normally nourished animals.

In some preliminary experiments on 1-month-old rats, it was found that an infection of the nature wanted could be produced in rats by ingestion of 20 drops of a 20 hours' broth culture of a certain Breslau strain (paratyphoid bacillus), described by Moltke (1926). The Peyer's patches and mesenteric glands were infected 1 day after the inoculation; the liver, the spleen and sometimes some peripheral lymph-glands were infected after 3 days. This extension of the lesion persisted practically without any change for about 10 days, when the infection began to subside slowly; as a rule it had completely passed away 24–28 days after the inoculation. In none of 25 rats which were examined at various stages of the infection was growth obtained in cultures from the heart's blood; none of the animals died spontaneously after the aforementioned dose (20 drops).

Besides, additional experiments showed that the size of the dose, within certain limits, was of no demonstrable consequence. Thus the features of the infection after ingestion of 40 drops of a culture were in every respect like the above-mentioned findings after ingestion of 20 drops.

The first experiment was aimed to show whether the normally non-fatal dose of 20 drops of a 20 hours' broth culture of the Breslau bacillus by mouth might produce a fatal infection in rats suffering from vitamin A deficiency.

A litter of six and one of eight young pied rats, 1 month old, were put on experimental diet: each litter was divided equally, one half was on vitamin A-free diet, and the other half was on an altogether adequate diet.

The technique in these vitamin experiments is the one that has been worked out in the Hygienic Institute of the University, where the under-noted diets for these experiments have been prepared. Details of this technique are given in papers by Fridericia (1927), Fridericia and Gudjonsson (1927), Freudenthal (1927), Holm (1924), and Gudjonsson (1926).

These diets have the following composition:

Vitamin A-free diet			Control diet		
200	parts	casein (vitamin A removed)	200	parts	casein
50	,,	salt mixture	50	,,	salt mixture
150	,,	lard	150	,,	butter-fat
550	,,	rice starch	550	,,	rice starch
50	,,	dried yeast	50	,,	dried yeast

The *salt mixture* consists of:

10·38	parts	sodium chloride
15·96	,,	magnesium sulphate
20·82	,,	primary sodium phosphate
57·24	,,	secondary potassium phosphate
78·00	,,	calcium lactate
32·40	,,	primary calcium phosphate
7·08	,,	ferric citrate

The *lard* was rendered from Danish leaf-lard, poured into a large retort and heated for 12 hours at 102° C. under aeration.

The *butter* was heated and poured into a separating funnel, the water and most of the casein being drawn off, whereafter the butter-fat was filtered through a double gauze filter.

The *rice starch* was from E. Remie et Cie, Antwerp. It was not purified, but ground fine.

The *casein* was finely powdered, raw, acid-precipitated casein, boiled with 96 per cent. alcohol for 1 hour, then pressed dry. This procedure was repeated three times. After the last boiling, the casein was completely dried at room temperature and then placed in an oven on flat trays for 24 hours at 100–102° C.

The *yeast* (top yeast) was autolysed for 24 hours at 37° C., dried on flat glass trays at about 30°, and pulverised by grinding.

The young rats were placed in galvanised wire cages with rather wide meshes, to prevent coprophagy.

Nos. 1–7 were put on the vitamin A-free diet, Nos. 8–14 on the control diet. Hardly 6 weeks later, all the rats on the A-free diet were rather out of sorts, all showing more or less pronounced xerophthalmic changes.

At this time, the average weight of Nos. 1–7 was 70 grm. and of Nos. 8–14 was 166 grm.

The rats were now infected, Nos. 1–7 getting 20 drops each, and Nos. 8–14 40 drops each of a 24 hours' Breslau broth culture, on a little cube of wheaten bread. The animals, especially those on vitamin A-free diet, pounced at once upon the bread cube, which they devoured voraciously.

In the course of the infection, the control animals showed next to no clinical reaction, but the rats on A-free diet were plainly very much affected and, after 8–10 days, sat crouching in a corner of the cage, their heads drawn down toward the abdomen—also, after about 10 days, they all had haemorrhagic diarrhoea which soiled their abdomen and hind legs.

The autopsy technique is the same as that employed by Ørskov and collaborators, and the bacteriological findings are verified after the same principles as mentioned in a previous paper (Lassen, 1929). As to the intestinal findings, only the positive cultures from the lowest section of the intestine were verified.

Table I gives the bacteriological autopsy findings with regard to the presence of Breslau bacilli in the organs at the time of autopsy, demonstrated by cultures from these organs.

The experiment shows distinctly a lowering of the resistance in the rats which had no vitamin A in their food. For there is hardly any doubt that Nos. 1, 3, 7, 2, and 6 died of the infection and not of vitamin deficiency. On the 15th day, the three control animals proved to be only slightly infected, as Breslau bacilli could be cultivated only from the mesenteric glands and (in two cases) Peyer's patches, while all the other organs yielded no growth of the inoculated micro-organism.

The rats on vitamin A-free diet that survive the climax of the infection,

Table I.

Rat No.	1	3	7	2	6	8	9	10	4	5	11	12	13	14
Diet	A-	A-	A-	A-	A-	N	N	N	A-	A-	N	N	N	N
Death	†	†	†	†	†	K	K	K	†	K	K	K	K	K
Time from infection to death (days)	9	13	13	14	15	15	15	15	19	29	29	29	29	29
Cultures from:														
Blood	–	+	+(+)	– – –	–	–	–	–	–	–	–	–	–	–
Liver	+ +(+)	+ + +	+ + +	+ + +	+(+)	–	–	–	–	(+)	–	–	–	–
Spleen	+ + +	+ + +	+ + +	+ + +	+	–	–	–	–	(+)	–	–	–	–
Lungs	+(+)	+ + +	+ + +	+ + +	+	–	–	–	–	–	–	–	–	–
Submaxillary lymph gland	+ + +	+ + +	+ + +	+ + +	+ + +	–	–	–	+ + +	+	–	–	–	–
Axillary gland	–	–	–	.	–	–	–	–	–	–	–	–	–	–
Inguinal gland	–	–	–	+ + +	–	–	–	–	–	–	–	–	–	–
Bladder	–	–	–	–	–	.	.	.	.	–	.	.	.	.
Peyer's patches	.	.	.	–	.	+ +	–	+ +	.	.	–	–	–	–
Mesenteric gland	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +(+)	+ +	+(+)	–	–	–	–
Contents of:	–													
Stomach	–	(+)	–	+ + +	–	–	–	–	–	–	–	–	–	–
Small intestine I	–	+ +(+)	–	+ + +	–	–	–	–	–	–	–	–	–	–
Small intestine II	–	–	+ +	+ + +	–	–	–	–	–	–	–	–	–	–
Small intestine III	–	–	–	+ + +	–	–	–	–	–	–	–	–	–	–
Caecum	–	–	–	–	–	–	–	+	–	–	–	–	–	–
Rectum	–	–	–	–	–	–	–	–	+ +	–	–	–	–	–

– signifies that the Breslau bacillus is not isolated from the organ in question.
+ signifies growth of the Breslau bacillus in culture from the respective organs. The amount of colonies in a culture is indicated by number of +.
Diet: A- means vitamin A-free; N stands for normal control diet.
Death: † signifies that the animal died spontaneously; K that it was killed.

however, are apparently able to overcome the infection like the controls; but the final elimination of the infecting bacilli seems to take longer in them than in the controls.

Just as the base of the tongue was the most frequent site of spontaneous infection in Green and Mellanby's material, so the infection in these rats was most frequently demonstrated in the regional lymph glands at the base of the tongue, the submaxillary lymph glands, as these glands were infected in all seven animals on vitamin A-free diet. In all the cases where cultures were made from the bladder there was growth of colon bacilli, but no growth of the Breslau bacillus. As all the control rats were killed, there is no way of knowing how long they might otherwise have lived, but there is nothing to suggest that they would have died as a result of the infection.

Rats which have died of vitamin A deficiency often show inflammatory changes in the intestinal tract, together with atrophy of the intestinal mucosa and lymphatic structures. One might therefore think that the lowered resistance to infection by mouth in rats fed on a vitamin A-free diet would be due to a reduction in the effectivity of the first barrier against bacterial invasion of the organism. This might result in a much larger percentage of the ingested bacteria penetrating the intestinal mucosa and thus gaining a foothold there. This would happen more easily in rats on vitamin A-free diet than in the rats on normal diet.

As the question of quantitative differences in bacterial invasion of the intestinal mucosa would most likely be very difficult to settle, it was considered more expedient to investigate the resistance to parenteral infection through subcutaneous inoculation.

Preliminary experiments were made in which increasing doses of 24 hours' broth cultures of the Breslau bacillus were injected subcutaneously into the abdominal wall of rats, and it was found that 0·25 c.c. of such a culture per 70 grm. of body-weight was fatal to rats on normal diet. It was then decided to employ a dose of approximately 0·1 c.c. of a 20 hours' broth culture per 60 grm. of body-weight. For the sake of accuracy of dosage, the broth culture was diluted 1 : 10.

Of a litter of eleven young pied rats, 1 month old and each weighing

Table II.

No.	Weight before put on special diet (grm.)	Weight 26 days later (grm.)	Subcutan. dose diluted 1 : 10 (c.c.)
A-free diet			
15	41	58	1·0
16	42	55	0·9
17	41	65	1·1
18	43	61	1·0
19	43	58	1·0
20	41	58	1·0
Normal diet			
21	40	96	1·6
22	42	88	1·5
23	42	112	1·9
24	43	114	1·9
25	44	118	2·0

https://doi.org/10.1017/S0022172400010469 Published online by Cambridge University Press

between 40 and 44 grm., six (Nos. 15–20) were put on vitamin A-free diet, five (Nos. 21–25) on normal diet.

26 days later all the rats on vitamin A-free diet, except one, had distinct eye symptoms; and they had all stopped growing.

The needle is passed into the abdominal wall, midway between fore and hind legs, and directed toward the left axilla.

Table III gives the bacteriological autopsy findings in this experiment. In the cases with positive blood culture, only these colonies are identified, together with the bacteria isolated from the lower section of the intestine giving positive culture. In Nos. 24 and 25 the Breslau bacillus was identified in cultures from all the organs and from some portions of the gastro-intestinal content.

The experiment shows that the injected dose has been large enough, as two of the animals on normal diet (Nos. 21 and 22) died 2 days after the inoculation, showing the same extension of the infection as was observed in all the animals on vitamin A-free diet. 7 days after the injection, all six rats on vitamin A-free diet are dead, while only two out of five rats on control diet have been overcome by the infection. No. 23 was killed on the 7th day for comparison with two rats on vitamin A-free diet that were dead that day; at this time it showed no clinical signs of the infection, although the autopsy revealed bacteriaemia. The table does not show any great deal of difference between the three animals that died on the 7th day, but the number of colonies in the blood cultures from these animals differs considerably more than may be expressed through the plus graduation. Count of the colonies in the blood cultures showed:

No. 19	...	...	1000 colonies
No. 20	...	... *ca.* 350	,,
No. 23	...	... 34	,,

It is most likely that No. 23 would have overcome the infection, just as Nos. 24 and 25. The autopsy on Nos. 24 and 25, which were killed on the 16th and 30th days respectively, showed a gradual decrease of the infection. In No. 25 cultures from the blood, liver and spleen showed no growth, while the Breslau bacillus was still obtained from most of the glands.

In the following experiment the subcutaneous inoculation dose was reduced to 0·5 c.c., 1/10 diluted, 24 hours' broth culture per 60 grm. of body-weight. The experiment comprises seventeen young rats, 1 month old, belonging to three litters. Nine of these animals (Nos. 26–34) were put on vitamin A-free diet, eight (Nos. 35–42) on normal diet. The litters were divided as shown in Table IV.

After a preliminary period of 36 days, all the animals on vitamin A-free diet had more or less pronounced eye symptoms; some of them had stopped growing, others were still gaining in weight but very slowly.

Table V gives the bacteriological autopsy findings as to the presence of the Breslau bacillus. The positive findings are verified in the same way and after the same principles as in the preceding experiment.

Table III.

Rat No.	17	15	21	22	18	16	19	20	23	24	25
Diet	A-	A-	N	N	A-	A-	A-	A-	N	N	N
Death	†	†	†	†	†	†	†	†	K	K	K
Time from infection to death (days)	1	2	2	2	3	5	7	7	7	16	30
Cultures from:											
Blood	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+(+)	-	-
Liver	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+(+)	-
Spleen	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	-
Lungs	+ + +	+ + +	+ + +	+ + +	.	+ + +	+ + +	+ + +	+(+)	+ +	+(+)
Submaxillary lymph gland	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ + +
Right axillary gland	+ + +	+ +	+ + +	+(+)	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+
Left axillary gland	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	(+)
Right inguinal gland	+ + +	+	+ + +	+ +(+)	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	-
Left inguinal gland	+ + +	+	+ + +	+ + +	+ + +	+ +	.	+ + +	+ + +	.	+ +
Peyer's patches	+ + +	.	-	+	.	+ + +	+ + +	+ +	+ + +	+ +(+)	+
Mesenteric gland	+ + +	+ + +	+ +(+)	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +(+)	+ +
Contents of:											
Stomach	+	+ + +	-	+(+)	.	+(+)	+(+)	+	-	+	-
Small intestine I	+ + +	+ + +	-	-	.	+ +	+(+)	-	-	-	-
Small intestine II	-	+ + +	-	-	.	+ +	-	-	-	-	-
Small intestine III	-	+ +(+)	-	-	.	-	-	-	-	+(+)	-
Caecum	+ + +	+ +	-	-	.	-	-	-	+ +	+ + +	-
Rectum	+ + +	+ + +	-	-	.	-	-	-	+	-	-

Table V.

No. A-free diet	Litter	Weight before put on special diet (grm.)	Weight 36 days later (grm.)	Subcutan. dose diluted 1 : 10 (c.c)
26	5	68	94	0·80
27	5	69	90	0·75
28	5	63	86	0·70
29	6	60	81	0·675
30	7	43	78	0·65
31	7	45	88	0·75
32	7	38	75	0·625
33	7	40	68	0·575
34	7	37	60	0·50
Normal diet				
35	5	73	177	1·50
36	5	71	173	1·45
37	6	82	188	1·55
38	6	52	135	1·15
39	7	39	137	1·15
40	7	40	148	1·25
41	7	44	171	1·45
42	7	38	133	1·15

This experiment shows more clearly than the preceding one a pronounced decrease of resistance in rats with symptoms of vitamin A deficiency. Within 4 days after subcutaneous inoculation with living Breslau bacilli, eight out of nine animals with dietary xerophthalmia have died, while eight animals on an adequate diet lived more than 37 days. No. 31 lived 22 days after the injection and has probably died of vitamin A deficiency; in this case no Breslau bacilli were found in the cultures from liver and spleen, these cultures being overgrown by proteus bacilli.

Autopsy of the control animals reveals the fact that the infection persists in the lymph glands, especially the regional glands, a very long time after the general infection has been overcome.

Summary.

Young normal rats are put on vitamin A-free diet and, after development of pronounced xerophthalmic symptoms, inoculated by mouth and by subcutaneous injection with Breslau bacilli (paratyphoid). The course of infection and bacteriological autopsy findings show a marked decrease in the resistance of these animals to this infection as compared with the findings in rats on adequate diet.

The experiments do not show any change in the *mechanism of infection* in pronounced vitamin A deficiency.

My most cordial thanks are due to Professor L. S. Fridericia, M.D., and to the Director of the State Serum Institute, Th. Madsen, M.D., for encouragement, advice and excellent working facilities.

Table V.

Rat No.	34	26	27	29	30	33	28	32	31	38	39	42	40	35	36	37	41
Diet	A-	A-	A-	A-	A-	A-	A-	A-	A-	N	N	N	N	N	N	N	N
Death	†	†	†	†	†	†	†	†	†	K	K	K	K	K	K	K	K
Time from infection to death (days)	1	2	2	2	3	3	4	4	22	37	37	38	38	46	46	67	67
Cultures from:																	
Blood	+++	++	++(+)	+++	+++	+(+)	+++	++	–	–	–	–	–	–	–	–	–
Liver	+++	.	.	.	.	.	.	.	?	–	–	–	–	–	–	–	–
Spleen	+++	.	.	.	.	.	.	.	?	–	–	–	–	–	–	–	–
Lungs	+++	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–	–
Submaxillary lymph gland	+++	.	.	.	.	.	.	.	+++	+	–	++(+)	–	–	++(+)	–	–
Right axillary gland	+++	.	.	.	.	.	.	.	+++	–	–	–	–	–	–	–	–
Left axillary gland	+++	.	.	.	.	.	.	.	+++	–	++	–	(+)	–	–	–	–
Right inguinal gland	+(+)	.	.	.	.	.	.	.	+++	++	–	+(+)	++	–	–	–	–
Left inguinal gland	+++	.	.	.	.	.	.	.	+++	++	–	–	–	+(+)	+	–	++
Peyer's patches	.	.	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–
Mesenteric gland	–	.	.	.	.	.	.	.	+++	–	–	–	++	–	–	–	–
Contents of:																	
Stomach	–	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–	–
Small intestine I	–	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–	–
Small intestine II	–	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–	–
Small intestine III	–	.	.	.	.	.	.	.	–	–	+	+	–	–	–	–	–
Caecum	–	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–	–
Rectum	–	.	.	.	.	.	.	.	–	–	–	–	–	–	–	–	–

REFERENCES.

BLOCH, C. E. (1917). *Ugeskrift for Læger*, **79**, 309.
CRAMER, W. and KINGSBURY, A. N. (1924). *Brit. J. Exp. Path.* **5**, 300.
FREUDENTHAL, P. (1927). [Experimental rickets.] Copenhagen: Levin and Meinksgaard.
FRIDERICIA, L. S. (1927). *Physiol. papers dedicated to Prof. Aug. Krogh*, 790.
FRIDERICIA, L. S. and GUDJONSSON, S. V. (1927). *Bibl. for Læger*, **119**, 790.
GREEN, H. N. and MELLANBY, E. (1928). *Brit. Med. J.* **2**, 691.
GUDJONSSON, S. V. (1926). *Amer. J. Physiol.* **75**, 533.
HOLM, E. (1924). *Bibl. for Læger*, **116**, 590.
LASSEN, H. C. A. (1929). *Zeitschr. f. Immunitätsf.* **63**, 110.
MOLTKE, O. (1926). *Acta path. et microbiol. scand.* **3**, 711.
ØRSKOV, J., JENSEN, K. A. and KOBAYASHI (1928). *Zeitschr. f. Immunitätsf.* **55**, 34.
ØRSKOV, J. and MOLTKE, O. (1928). *Ibid.* **59**, 357.
ØRSKOV, J. and SCHMIDT, A. (1928). *Ibid.* **55**, 69.
PRITCHETT, I. (1927). *J. Exp. Med.* **46**, 557.
VERDER, E. (1928). *J. Infect. Dis.* **42**, 589.
WEBSTER, L. T. and PRITCHETT, I. (1924). *J. Exp. Med.* **40**, 397.
WERKMANN, C. H. (1923). *J. Infect. Dis.* **32**, 247.
—— (1923). *Ibid.* **32**, 255.
—— (1923). *Ibid.* **32**, 263.
WERKMANN, C. H., BALDWIN, F. M. and NELSON, V. E. (1924). *Ibid.* **35**, 549.

(*MS. received for publication* 21. XII. 1929.—Ed.)