VOLUME XXXIII AUGUST, 1933 No. 3

THE EFFECTS OF COLLOIDAL SILICA ON EXPERIMENTAL TUBERCULOSIS IN GUINEA-PIGS.

BY PROFESSOR S. L. CUMMINS, C.B., C.M.G., M.D.
AND DR CICELY WEATHERALL, M.D., D.P.H.

From the Tuberculosis Department, Welsh National School of Medicine, Cardiff.

GYE AND KETTLE (1922), from a study of the effects of subcutaneous inoculation of silica alone, tubercle bacilli alone and tubercle bacilli with silica, arrived at the conclusion that there is, after the introduction of tubercle bacilli with silica, "a much greater local reaction than with an infection of tubercle bacilli alone and, further, that general dissemination is earlier and more active." As a result, "a small dose of bacilli becomes a dose of considerable magnitude and, inasmuch as an important factor in determining an infectious process is the number of organisms introduced, a simple explanation is forthcoming of the effect of silica upon such tuberculous lesions as we have described."

In view of the experiments of Krause and Willis (1924) in which they have shown that the inflammatory reaction produced by the inoculation of tubercle bacilli into the skin of allergic guinea-pigs is associated with a retardation of the dissemination of the bacilli from the site of inoculation, it struck us that the more rapid diffusion of bacilli found by Gye and Kettle to be associated with the inflammation caused by silica was a matter of great interest and meriting further study on slightly different lines.

We decided to study the local and general effects of intradermal and intratesticular inoculations, sites from which diffusion of bacilli is normally less rapid than from the subcutaneous areolar tissue; and also the general effects of subcutaneous inoculations. We proposed to use, as one of the criteria of rapidity of dissemination of bacilli, the length of survival of the inoculated animals after infection. Further, we decided not to repeat the inoculations of silica into the lesions, as was done by Gye and Kettle, but to confine our observations to following up the effects of a single inoculation of each of the mixtures tested. We anticipated that, if the presence of silica along with tubercle bacilli in the tissues led to a small dose of bacilli soon attaining to such numbers of germs as to be a dose of considerable magnitude, this fact should be reflected in a marked shortening of the survival period in the inoculated animals, as compared with the "controls" as well as with greater severity of the lesions produced.

In the hope of throwing additional light on the influence of increased local

pullulation through silica, we decided to give graded doses of counted emulsions of tubercle bacilli to those animals set aside for "survival" experiments. It may be said at once that this grading of dose failed to afford any information of value, the survival periods of the "control" animals not corresponding to the doses administered. Accordingly, in the last series of experiments, on intratesticular inoculation, the attempt at grading of dose was abandoned and all the animals received, approximately, the same number of tubercle bacilli.

Experiment I.

Production and examination of early lesions following intradermal inoculations.

Four guinea-pigs were used, three of them, Nos. 1958, 1959 and 1964, being previously uninfected animals from stock, and the fourth, No. 1918, an animal infected in the testing of pus from a cold abscess about 6 weeks previously and now showing definite signs of tuberculosis.

It was thought advisable to include one infected animal, as it seemed possible that its allergic tissue reaction to the tubercle bacilli might introduce an interesting factor for comparison with the findings in the non-allergic animals. Areas of skin were depilated on either side of the vertebral column in each of these animals.

A 25-days' old egg medium culture of T.B. Constantanides [human] was emulsified in distilled water, filtered through cotton-wool, centrifuged to remove clumps and counted against a standard Staphylococcus emulsion, being estimated to contain approximately 1,376,000,000 tubercle bacilli per 1 c.c.

Each animal was inoculated intradermally on the left side with a mixture of equal parts of this tubercle emulsion and colloidal silica solution, prepared in the same way and of the same strength as that used by Gye and Kettle: sufficient being introduced to raise a "wheal" of about the size of a threepenny piece. A volume of about 0·2 c.c. was used in each case for this purpose.

On the right side, two intradermal inoculations were given of similar volume, the anterior one consisting of tubercle emulsion alone, diluted with an equal quantity of sterile distilled water, while the posterior inoculation was of colloidal silica mixed with an equal volume of distilled water but free from bacilli. We were thus in a position to compare the effects of silica sol. alone, tubercle emulsion alone and tubercle emulsion along with silica sol. These inoculations were made on 19. xi. 30. The size of the "reactions" (in millimetres) was measured across on 21. xi. 30 and the results are set out in Table I.

Table I.

Guinea-pig serial No.	Left side. Silica + T.B. (mm.)	Right side. Ant. T.B. alone (mm.)	Right side. Post. silica alone (mm.)
1958	9·5	5·5	8·0
1959	12·0	5·0	13·0
1964	7·0	6·0	8·0
1918	16·0	16·0	13·0

In every case the inoculations of silica, whether with or without tubercle bacilli, had produced a raised, red circumscribed swelling of characteristic appearance, whereas the inoculations of tubercle bacilli alone, in the three non-allergic animals at least, had led to little or no inflammatory reaction at 48 hours, and could only be measured with difficulty as a slightly elevated induration differing but little from the surrounding healthy skin.

In the allergic animal, guinea-pig No. 1918, the picture was quite different. Here the inoculation of tubercle bacilli alone had produced an inflamed and elevated reaction just as large as that produced by silica sol and tubercle bacilli together; whereas the silica sol. alone, without the amplification resulting from an allergic reaction superposed on the irritation of the silica, had led to an elevated area less extensive than either of those in which tubercle bacilli had been used.

The animals were killed for examination on the following dates:

Guinea-pig No. 1959.	21. xi. 30.	48 hours after inoculation.
Guinea-pig No. 1958.	25. xi. 30.	6 days after inoculation.
Guinea-pigs. Nos. 1964 and 1918.	1. xii. 30.	12 days after inoculation.

The inoculated portions of skin, with their elevated areas of inflammation, were carefully dissected off, placed to harden in formol saline, divided across the middle with the intention of exposing comparable parts of the lesions, blocked in paraffin, and cut into sections for microscopic examination. At the same time, cultures from the heart blood of guinea-pigs Nos. 1959 and 1958 were put up in veal broth according to a method devised by Professor Dreyer who had kindly demonstrated his technique to one of us. In neither case did these blood cultures prove positive; from which it may be inferred that the diffusion of bacilli into the general circulation from these intracutaneous lesions had, up to the sixth day, been minimal.

Microscopic examination of lesions.

Guinea-pig 1959 (killed 48 hours after inoculation). There was a sharp contrast between the lesions produced by colloidal silica solution, with or without tubercle bacilli, and the lesion produced by tubercle bacilli alone. In the former, it was easy to find the exact point of inoculation which was visible under the low power; while in the latter, the cellular reaction was so slight that low-power examination left the observer uncertain as to whether any definite lesions were present.

In sections stained with Ziehl and methylene blue, again, the finding of tubercle bacilli was a much simpler matter in the lesion produced by silica and tubercle bacilli together than in that produced by tubercle bacilli alone, owing to the guidance afforded by the marked cellular reaction in the former. Apart from this aid to the discovery of the bacilli, however, the arrangement and numbers of the bacilli in the two sections were not markedly dissimilar. In both, the bacilli were, for the most part, still free and usually in clumps, being rigidly restricted in distribution to a small area so that nearly all the "positive" fields were found in close succession after a long preliminary search

through many completely negative fields. Although the bacilli were more numerous in the "silica with tubercle" lesion, the differences were of an order which might be due to the accident of having hit off a more favourable part of the lesion in the one section than in the other or to a retention of bacilli through the inflammatory reaction produced by the silica.

The lesion produced by "silica alone" was, save for the absence of bacilli, indistinguishable histologically from that produced by "silica with tubercle"; a small central abscess-like area occupied by a collection of cells, chiefly polymorphonuclear leucocytes with a few lymphocytes and rather numerous large mononuclear cells of the "macrophage" type while a few eosinophile cells also were present; and a peripheral zone in which the tissues of the cutis were infiltrated with numerous cells of the types above described. Many of the cells showed signs of degeneration and pyknotic nuclei were common.

Guinea-pig No. 1958 (killed 6 days after inoculation). In this animal the lesion produced by tubercle bacilli alone was, after the lapse of 6 days, more cellular and easier to find than the 48-hour lesion in guinea-pig No. 1959, but the arrangement of the bacilli had altered considerably. All were now intracellular and their distribution was much less localised than in the 48-hour lesions. It was evident that they were being carried along tissue spaces and lymph channels in the interior of phagocytes.

The lesions produced by silica solution with tubercle and by silica solution alone were still of much the same type as those seen after 48 hours except that some of the cells in the surrounding tissue spaces were elongated and flattened, suggesting an early fibroblastic change.

In spite of the clearer visibility of the areas of lesion produced by tubercle bacilli alone in guinea-pig No. 1958 as compared with those found in guinea-pig No. 1959, the bacilli appeared much less numerous and, in some of the sections, were so rare as to be very difficult to find.

Guinea-pig No. 1964 (killed 12 days after inoculation). In the sections from this animal the inflammatory character of the lesion produced by tubercle bacilli alone was fairly well marked owing to a definite cellular reaction. That following the inoculation of silica alone was characterised by a tendency towards a clearing up of the inflammatory and cellular exudate and by the appearance of fibroblasts in the surrounding tissue spaces, apparently developing from the cells seen in these situations in the 48-hour and 6-day lesions.

The lesion produced by silica solution combined with tubercle bacilli was still, in this 12-day stage, markedly cellular with much the same characters as those in the animals killed at earlier dates. While the bacilli appeared to be very much reduced in number in the lesion produced by tubercle bacilli alone, they were, in the "silica and tubercle" lesion, more numerous in some, at least, of the sections examined.

Guinea-pig No. 1918 (allergic animal, killed 12 days after inoculation). In this guinea-pig the lesions were similar to those described in guinea-pig

No. 1964 except that the cellular reaction was still more marked in the lesions containing tubercle bacilli. That following the inoculation of silica alone was less advanced in the direction of fibroblastic change than in guinea-pig No. 1964.

In attempting to decide from the examination of the intradermal lesions in these animals whether or not the addition of colloidal silica solution to the tubercle emulsion had acted as an adjuvant to the development of the tubercle bacilli in the tissues we were faced with several difficulties.

It was clearly true that, in the early stages in the non-allergic guinea-pigs, there was less inflammatory change around the lesion produced by tubercle bacilli alone and, therefore, a greater freedom for the distribution of the bacilli along the tissue spaces and away from the lesion. Where silica sol was introduced along with the bacilli, the latter were, perhaps, more likely to be retained *in situ* owing to the inflammatory response to the silica and might thus seem more numerous locally, giving a spurious appearance of increase as compared with the purely tuberculous lesion. Again, in the 48-hours' lesion, the cellular infiltration caused by the silica afforded an easy guide to the bacilli which were, on the other hand, difficult to find in the case of the inoculation of tubercle bacilli alone. Once found, however, the bacilli seemed to be only slightly less numerous in the "pure tubercle" than in the "silica with tubercle" lesions.

We gained the impression that the allergic reaction in guinea-pig No. 1918 was accompanied to a greater extent than the reaction to silica by a tendency for the bacilli to remain localised. Some additional factor was present which interfered with the rapid dissemination of germs away from the site of inoculation, a conclusion in accord with the findings of Krause and Willis (1924).

Counting of tubercle bacilli in sections.

It seemed desirable to arrive at an approximation to a numerical expression of the differences in bacterial content in these lesions; and we decided to attempt to count the bacilli visible in the sections.

The ideal method of counting all the bacilli visible in serial sections through the various lesions produced in our experiments would have involved very much longer hours of work than we were in a position to devote to it. Instead, we attempted the much less satisfactory method of dividing the blocks as nearly across the centre as possible and counting the bacilli in what we hoped might prove to be corresponding parts of the lesions to be compared.

With a square counting obturator in the ocular and using a 1/12 in. o.i. lens, we found that the complete examination of a section involved the search for bacilli in from 300 to 500 fields, each field of a 4μ section requiring an exploration of several distinct focal planes.

Table II records our results as to the total number of "positive" fields, the total number of tubercle bacilli and the number of bacilli per "positive" field in the sections compared. The "negative" fields are left out of consideration.

Table II. *Results of enumeration of tubercle bacilli in sections.*

Guinea-pig serial No.	Time after inoculation		Number of "positive" fields	Total number of tubercle bacilli	Tubercle bacilli per "positive" field
1959	48 hours	S.T.	77	4001	52
		T.	82	2011	24
1958	6 days	S.T.	24	107	4·5
		T.	31	373	12·0
1964	12 days	S.T.	42	178	4·2
		T.	Only a few bacilli found in the sections examined		
1918 (allergic)	12 days	S.T.	38	89	2·3
		T.	74	700	9·4

S.T. = silica sol. with tubercle bacilli. T. = tubercle bacilli alone.

Owing to the difficulty of being sure that the portions of the sections compared actually correspond, we regard these findings as approximations only, but they suggest that, whether inoculated with or without silica, the bacilli are locally most numerous shortly after infection and that they tend, later, to disseminate outside the area of the inoculation. In the allergic animal, however, there is seen to be evidence of considerable localisation even 12 days after inoculation.

The observations suggest that, whatever the cause, the tubercle bacilli, in the animal killed 48 hours after inoculation, were about twice as numerous in the lesion produced by tubercle bacilli with silica as in the lesion produced by tubercle bacilli alone.

Experiment II.

Survival periods of guinea-pigs after intradermal inoculation of graded doses of tubercle bacilli; with and without colloidal silica.

An emulsion was prepared in sterile normal saline solution of a human strain, "T.B. Constantanides," which was counted and found to contain approximately 200,000,000 bacilli per 1 c.c. The culture was from an egg medium slope and was over 2 months old. This emulsion was diluted in tenths in two series each of four strengths of approximately 20,000,000, 2,000,000, 200,000 and 20,000 bacilli per 1 c.c.; series A being diluted with saline, series B with colloidal silica solution.

Intradermal inoculations, each of a volume of approximately 0·1 c.c., were administered to eight guinea-pigs as recorded in Table III.

Table III.

Series A. Guinea-pigs (tubercle bacilli in saline)	Series B. Guinea-pigs (tubercle bacilli in silica sol.)	Approx. numbers of bacilli
No. 1871	No. 1868	2,000,000
No. 1872	No. 1867	200,000
No. 1873	No. 1869	20,000
No. 1874	No. 1870	2,000

Forty-eight hours after inoculation, none of the animals in series A showed any appreciable change at the site of inoculation. All those in series B, how-

ever, presented local swellings which were visible and palpable as well-defined red infiltrated areas about the size of buck-shot. A week later, series A was still without local manifestations, while those in series B were as before but paler and less inflamed. No glands were as yet palpable in the inguinal regions in either group.

Fourteen days after the injections all the animals in series A presented small raised inflammatory swellings at the inoculation sites, the corresponding inguinal glands being palpable in guinea-pigs Nos. 1871 and 1872 while no glands were to be felt in Nos. 1873 and 1874.

At this date, in series B, guinea-pig No. 1869 was found dead, with a generalised peritonitis of non-tuberculous origin. The remaining three animals showed each a dry non-inflamed lump at the site of inoculation; while it was questionable whether or not there was slight swelling of the inguinal glands. These animals were kept under observation until they died of tuberculosis; except that two of them, Nos. 1871 and 1872, were killed, when *in extremis*, at the end of the 22nd week. The survival periods, in weeks, are recorded in Table IV.

Table IV.

Series A (T.B. alone)		Series B (T.B. + silica)	
Guinea-pig Nos.	Survival in weeks	Guinea-pig Nos.	Survival in weeks
1871 (killed)	22	1868	17
1872 (killed)	22	1867	17
1873	15	1869 (intercurrent infection)	2
1874	18	1870	15
Average (weeks)	19·2		16·3 (excluding 1869)

It will be seen that the average period of survival in series A was longer than that in series B and might have exceeded it by a day or two more if Nos. 1871 and 1872 had been left to die.

All the animals of both series, save No. 1869, showed the lesions of generalised tuberculosis at post-mortem examination.

EXPERIMENT III.

Survival periods after subcutaneous inoculations.

With the idea that a sharper contrast might be elicited with subcutaneous injections, this experiment was repeated, the same strain of bacillus being used from an egg culture of the same age, and standardised to the same strength, approximately 200,000,000 per 1 c.c.

Two series of dilutions, A and B, without and with colloidal silica solution, were made as before, the eight guinea-pigs, however, being inoculated subcutaneously in the left inguinal region instead of intradermally.

The survival periods in these guinea-pigs are recorded in Table V.

Table V.

Series A (T.B. alone)		Series B (T.B. + silica)	
Guinea-pig Nos.	Survival in weeks	Guinea-pig Nos.	Survival in weeks
1913	17	1909	20
1912	28	1908	19
1915	16	1911	10
1914	22	1910	23
Average (weeks)	20·7		18·0

In this series, in which all the animals died with chronic generalised tuberculosis, the average survival period was, as in the previous series, slightly less in series B, the group in which silica was injected along with tubercle bacilli, than in series A in which no silica was used.

Experiment IV.

Intratesticular inoculations.

On 17. xi. 31, ten male guinea-pigs were selected and divided into three groups for inoculation experiments as follows:

Group X, receiving silica sol. alone, consisted of two guinea-pigs, Nos. 2312 and 2315.

Group A, receiving tubercle bacilli alone, consisted of four guinea-pigs, Nos. 2314, 2316, 2317 and 2318.

Group B, given tubercle bacilli with silica sol., included four guinea-pigs, Nos. 2309, 2310, 2311 and 2313.

All the animals in groups A and B received into the right testicle approximately 40,000,000 of a 4-weeks old egg culture of T.B. Singh, a "human" strain derived from the sputum of a case of pulmonary tuberculosis. The bacilli were suspended in distilled water for group A and in silica sol. in group B. The concentration of silica sol. was the same in group X as in group B. In all cases, the volume of fluid injected was 0·2 c.c. and the left testicle was kept untreated for comparison.

On 19. xi. 31, all the animals showed swelling and hardness in the right testicles.

On 27. xi. 31, the effects of inoculation were noted as follows:

Group X, no swelling.

Group A, all the animals showed slight swelling and some hardness of right testicle.

Group B, all showed marked hardness and considerable swelling in right testicle.

It will be noted that the inflammation caused by silica sol. alone, which was definite in group X at 48 hours, had passed away by the tenth day; but the hardness and swelling caused by tubercle bacilli with silica in group B was now definitely greater than that resulting from tubercle bacilli alone in group A.

On 1. xii. 31, one animal from each group was killed for examination. That from group X, No. 2315, showed little or no change beyond, perhaps, a slight diminution in size of the right as compared with the left testicle. Sections of the right testicle showed some intertubular sclerosis.

Of the tuberculous animals, guinea-pig No. 2311, inoculated with tubercle bacilli and silica, showed much more local disturbance and more infection up along the vas deferens than guinea-pig No. 2317, inoculated with tubercle bacilli alone.

The other animals were allowed to survive until they died of tuberculosis, except guinea-pig No. 2312 of group X which, being non-infected, was killed after the death of the last of the inoculated animals. The survival periods of the latter, in days, are recorded in Table VI.

Table VI.

Series A (T.B. alone)		Series B (T.B. + silica)	
Guinea-pig No.	Survival period (days)	Guinea-pig No.	Survival period (days)
No. 2314	58	No. 2309	57
No. 2316	55	No. 2310	55
No. 2318	70	No. 2313	57
Average (days)	61		56

Here again the average survival period was slightly longer in the animals of group A (without silica), but in two out of three there was no difference.

In order to obtain a basis for comparison between the amount of tuberculosis found at post-mortem examination in these experiments, we decided to adopt the method of "Anatomical Study" used by Maver and Wells (1924) in a somewhat similar investigation.

"The degree of tuberculous involvement was estimated in the following manner: the four chief sites of tuberculosis were selected (lungs, spleen, liver and lymph nodes) and the extent of tuberculosis in each graded as follows: slight, 1; moderate, 2; marked, 3; extreme, 4." The gradings were added and averaged with the following results:

	Average marks
Intradermal inoculations:	
Series A (tubercle bacilli alone)	9·0
Series B (tubercle bacilli with silica)	12·3
Subcutaneous inoculations:	
Series A (tubercle bacilli alone)	12·5
Series B (tubercle bacilli with silica)	12·25
Intratesticular inoculations:	
Series A (tubercle bacilli alone)	9·6
Series B (tubercle bacilli with silica)	7·6

While the extent of tuberculosis was, on the whole, less in series A than in series B in the "intradermal" group, no real difference was noted in the "subcutaneous," nor do we feel certain that the differences were significant in any group.

It is questionable, too, whether the slightly shorter survival of the guinea-pigs inoculated with tubercle bacilli suspended in colloidal silica solution in

these experiments has any great significance. The "fluky" nature of the dates of death after the inoculations is shown by the fact that there was no correspondence, in the groups of animals inoculated with graded doses, between the size of the dose given and the period of survival after infection.

In the intratesticular experiment, the dose was larger and was not varied, all animals receiving an estimated 40,000,000 of bacilli. As a result the survival periods were shorter and are, therefore, expressed in days instead of in weeks. Here the lesions in lungs, liver, spleen and lymphatic glands were anatomically smaller than in the earlier experiments because there was not time for the generalised lesions to attain their full dimensions. Microscopic examination, however, proved that the type of lesion, though less advanced, was more acute and destructive, so that the marks do not quite express the severity of the disease in these animals.

Turning to the local lesions, these were decidedly more severe in series B (T.B. with silica) than in group A (T.B. alone). This fact is brought out in Table VII in which, using the method of Maver and Wells, marks are given up to a "possible" of four each for the extent of lesions in the testicles and epididymes.

Table VII.

Series A (T.B. alone)			Series B (T.B. + silica)		
	Marks for testicle and epididymal lesions			Marks for testicle and epididymal lesions	
Guinea-pig No.	Rt. test. and epid.	Lt. test. and epid.	Guinea-pig No.	Rt. test. and epid.	Lt. test. and epid.
2314	4	2	2309	6	2
2316	5	3	2310	5	0
2317 (killed)	4	0	2311	5	0
2318	4	1	2313	5	2
Average	4·3	1·5		5·2	1·0

It is to be noted that, in this experiment with intratesticular inoculation, the greater severity of the lesions in the right testicles and epididymes in series B (T.B. with silica) went with diminished generalisation of lesions into lungs, liver, spleen and glands and with less tendency to a spread of tuberculosis into the opposite testicle. Here the observations appear strongly to suggest a *localising* effect through the inflammatory action of the silica sol. in which the bacilli were suspended.

Discussion.

Our experiments confirm those of Gye and Kettle that there is a much greater local reaction after the inoculation of tubercle bacilli with silica sol. than after tubercle bacilli alone.

We are doubtful, however, as to whether our findings point to an earlier and more active dissemination of bacilli. It is true that the survival of the animals inoculated with tubercle bacilli alone was longer than of those given

similar doses of tubercle bacilli with silica. Of the twenty-one guinea-pigs allowed to survive until death from tuberculosis supervened or was imminent, the average survival period after inoculation with tubercle bacilli alone was 127 days; while the survival after tubercle bacilli with silica sol. was 101 days. A comparison of individual animals, however, suggests that the differences were slight and such as might well result from accidental factors. Again, the average severity of the tuberculous lesions found in the organs was in two out of three experiments actually greater in the animals given tubercle bacilli alone than in those given tubercle bacilli along with silica sol. If the average "marks" given according to the method of Maver and Wells be totalled for all three experiments, those accorded to group A (tubercle bacilli alone) come to 31·1 and those given to group B to 32·15, a difference so slight as to be insignificant.

On the other hand, the intratesticular inoculations suggest, on the whole, that the silica sol., while markedly increasing the local reaction, led to a diminution rather than to an increase of dissemination of tubercle bacilli throughout the organs.

If, as suggested by Gye and Kettle, "a small dose of bacilli" introduced along with silica "becomes a dose of considerable magnitude" and thus leads on to an earlier and more active dissemination of infection, we should have expected much sharper and more conclusive differences in survival periods and in the extent and severity of remote lesions.

We gather, however, from our experiments with "graded" doses of the tubercle strains used, that the numerical differences would have to be of an order greater than those employed, *i.e.* from 2000 to 2,000,000 bacilli, to elicit marked difference in survival period.

Possibly our failure to elicit this marked local pullulation of bacilli and its subsequent effects depended upon our giving a single inoculation only and not keeping up the effect by further local injections of silica; but to have done so would have rendered the comparison of our two groups unreliable, the one group having been subjected to repeated traumata and the other not.

On the other hand, we observed no such marked tendency to "localisation" through the inflammatory action of silica as was proved by Krause and Willis to be associated with the inflammatory reaction to reinfection with tubercle bacilli in allergic guinea-pigs; an interesting point worthy of further study, since it suggests that the arrest of tubercle bacilli at the site of injection in allergic animals is due to some other factor than the local inflammation. This observation is in keeping with the more recent findings of A. R. Rich (1930) and his co-workers who claim that a similar localisation of tubercle bacilli may occur, in immune but desensitised animals, without any inflammatory reaction to explain it.

Conclusions.

1. Our experiments confirm those of Gye and Kettle that the addition of silica sol. to tubercle bacilli leads, on inoculation into guinea-pigs, to a marked increase of local reaction and to a greater local pullulation of tubercle bacilli within the first few days after injection.

2. While we observed a shortening of the survival period in those animals given tubercle bacilli along with silica sol., as compared with the guinea-pigs infected with tubercle bacilli alone, the differences were slight and hardly to be regarded as significant. The adjuvant action of the silica sol. seems to be local and transitory, as is the local irritant action of silica sol. alone; and any tendency to a more rapid dissemination of bacilli would appear to be neutralised by the local fibrosis which undoubtedly follows the introduction of silica sol. into the tissues.

3. We regard these findings as of interest because they fall into line with the known fact that the tuberculosis death-rate, in industries involving exposure to silica dust, is high in late middle age and after many years of dust inhalation; not in the early years of exposure as should be the case if the silica determined an early generalisation of tuberculous infection.

This work has been greatly facilitated by a grant from the Medical Research Council for which we desire to express our thanks.

REFERENCES.

Gye, W. J. and Kettle, E. H. (1922). *J. Exper. Path.* **3**, 241.
Krause, A. K. and Willis, H. S. (1924). *Bull. Internat. Union against Tuber.* **1**, No. 3.
Maver, M. E. and Wells, H. Gideon (1924). *Amer. Rev. Tuberc.* **8**, 318.
Rich, A. Rice (1930). *Bull. Johns Hopkins Hosp.* **47**, 189.

(*MS. received for publication* 16. II. 1933.—Ed.)