THE RELATION OF THE TUBERCLE BACILLUS TO LYMPHADENOMA.

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In 1913, Fraenkel and Much[1] inoculated monkeys, guinea-pigs, rabbits and dogs intraperitoneally with large quantities of material obtained from two cases of Lymphadenoma, no accompanying tubercular disease being demonstrable. The material was broken down with antiformin, and as a result of the injections, the guinea-pigs died within three months. The authors state that besides finding extensive tubercular disease, there were hard, white nodules, the size of a cherry stone, on the serous coverings of the stomach and mesentery. Also, in addition to genuine tubercular disease of the lymphatic glands, they found giant cells, not of Langhans' type, as well as a stroma rich in fibriles, similar to what is found in Lymphadenoma. The nodules on the serous surfaces showed a picture corresponding to the terminal fibrous stage of a lymphadenomatous focus, with a scarcity of cellular elements.

The material utilised in later experiments was derived from ten cases of Lymphadenoma, verified as such microscopically, and which was apparently free from tubercular disease. In view of the importance of the animal experiments performed with this material, each case will be carefully considered, and remarked upon separately.

Case 1. Of four guinea-pigs inoculated with crushed gland, two died after four and five months of extensive tubercular disease, no mention being made of the other two animals. An additional four guinea-pigs inoculated with material treated with antiformin remained healthy.

Remarks. The two control animals both died of ordinary tubercular disease. When the lymphadenomatous material was treated with antiformin the animals survived, while we have just seen that the guinea-pigs inoculated in 1913, under similar conditions, did, in fact, develop tubercular disease.

Case 2. Again, four guinea-pigs were inoculated with crushed gland, but, unfortunately, no mention is made as to the result of the experiment. In view of the fact that French experimenters have found that dysentery bacilli, which had become avirulent, were rendered virulent by the utilisation of lactic acid, four guinea-pigs were injected with gland crushed up in lactic acid, and the animals subsequently developed extensive tubercular disease. The diseased organs from one of the animals were crushed up, and the pulped tissue injected into 18 guinea-pigs. When the tissue was treated with antiformin the six animals remained healthy, the other twelve developed tuber-

[1] Fraenkel, E. and Much, H. (1923). *Zeitschr. f. Hygiene u. Infektionskr.* xcix. 391.

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cular disease; those which received the gland plus lactic acid developing the disease earlier and more acutely than those injected with the gland alone.

Remarks. The fate of the control animals not being mentioned, all we can gather from this experiment is that the antiformin again apparently sterilised the inoculum, although it consisted of material taken from an animal dead with the ordinary lesions of tuberculosis.

Case 3. In this experiment 32 guinea-pigs were used, antiforminised material being injected into half of their number. The last-mentioned 16 animals remained healthy. In the second half of the experiment, four animals were injected with old gland, four with old gland plus lactic acid, four with recent gland, and four with recent gland plus lactic acid (see Table I for the results).

Remarks. Where the material was treated with antiformin the 16 animals again survived. Of the remaining 16 animals the six which died at an early date must be excluded; five showed no disease; and five developed disease, two of which proved to be suffering from ordinary tubercular disease, and from a third tubercle bacilli were eventually isolated. Also, it must be noted that of the five diseased animals, two were injected with the gland alone, and from one of these, at least, tubercle bacilli were isolated.

Case 4. Here the animal experiments gave no results as the gland was placed, by mistake, in 5 per cent. carbolic acid instead of 0·5 per cent. carbolic acid.

Cases 5 *and* 7. In each case two animals were inoculated with gland alone, and four with gland plus lactic acid. In case 4 (? 5) one of the animals, which received the gland plus lactic acid developed tuberculosis. In case 6 (? 7) two animals became tubercular, one having received the gland alone, and one the gland plus lactic acid.

Remarks. In the text of their paper cases 4 and 6 are referred to when presumably cases 5 and 7 are meant. If these two cases are considered together we see that of the four control animals one developed tubercular disease; of the four of each case where the material was treated with lactic acid, one animal also developed tubercular disease. Thus in these two cases the lactic acid showed no sensitising action.

Case 6. On the 18th of March, ten guinea-pigs were inoculated with fresh and treated gland which had been removed by operation. None of these animals showed any sign of disease seven months after injection. The patient died on the 8th of July of the same year and the three guinea-pigs inoculated with post-mortem gland developed tubercular disease, seemingly much to the surprise of the authors.

Remarks. The results of these animal experiments are evidently more against than in support of the thesis of Fraenkel and Much.

Case 8. The animal inoculations gave no result, due presumably, according to the authors, to the patient having had X-rays before removal of the gland.

Case 9. In this case two guinea-pigs were inoculated with crushed gland,

and five months later were found to be free from illness; two other animals inoculated with the gland plus lactic acid, developed tubercular disease.

Remarks. The experiment appears to show the sensitising action of the lactic acid.

Case 10. Only one guinea-pig was inoculated with the gland alone, and no infection resulted. Of the three animals which received the gland plus lactic acid, one remained healthy, one showed isolated liver and lung nodules, and one marked tuberculosis of the liver.

Remarks. Again in favour of the sensitising action of the lactic acid, although the use of only one control renders the experiment of little value.

In order that Fraenkel and Much's results as a whole can be seen at a glance, their animal experiments have been tabulated (see Table I).

Table I.

Case	Inoculations	Material	Died early	Nil	T.B.	? Lymphad.
1	4	Gl	2	.	2	.
	4	L	.	4	.	.
2	4	Gl	.	? 4	.	.
	4	L	.	.	4	.
	6	T.B.	.	.	6	.
	6	„ L	.	.	6	.
	6	„ A	.	6	.	.
3	8	A	.	8	.	.
	8	AL	.	8	.	.
	8	Gl	4	2	.	2
	8	L	2	3	2	1
4	?	.	.	.	.	.
5	2	Gl	.	2	.	.
	4	L	.	3	1	.
6	3	A.-m. Gl	.	3	.	.
	3	„ L	.	3	.	.
	2	„ A	.	2	.	.
	2	„ AL	.	2	.	.
	1	P.-m. Gl	.	.	1	.
	2	„ L	.	.	2	.
7	2	Gl	.	1	1	.
	4	L	.	3	1	.
8	?	.	.	.	.	.
9	2	Gl	.	2	.	.
	2	L	.	.	2	.
10	1	Gl	.	1	.	.
	3	L	.	1	2	.

Gl = Untreated gland. L = Gland treated with lactic acid. A = Gland treated with antiformin.

The conclusions that these authors draw from their experiments will now be given, together with my own criticism of their conclusions:

1. Lymphadenoma is a rare form of tubercular disease, but not rarer than some other forms of tubercular disease (for example, *Lupus erythematosus*).

Remarks. Lymphadenoma may be a rare form of tubercular disease, but I am of the opinion that the experimental proof offered is by no means sufficient to render such a conclusion justifiable. Tubercle bacilli were isolated on eight occasions from 11 specimens obtained from 10 cases. From four

cases only were tubercle bacilli isolated from some of the animals inoculated with the tissue plus lactic acid, while the control animals did not develop tubercular disease. But the number of control animals was in three out of these four cases not as great as was that of the animals inoculated with the gland plus lactic acid.

2. Special constitutional changes are necessary for its genesis. It is impossible to say what the changes are, as it is equally impossible to say the conditions governing the development of other tubercular diseases such as *Lupus vulgaris*, *Lupus erythematosus* or *Erythema induratum*.

Remarks. I agree with the authors that special constitutional changes are probably necessary for its genesis.

3. The fact that Lymphadenoma cannot be transmitted to animals is no argument against its being tubercular in nature, for the three diseases mentioned in the previous paragraph cannot be given to animals, and yet their tubercular nature is established with certainty.

Remarks. The fact that Lymphadenoma cannot be transmitted to animals is no argument against its being tubercular, but is it established beyond doubt that *Lupus erythematosus* is tubercular in nature? As regards *Lupus vulgaris*, the histological picture contains many of the characteristics of a tubercular process, and a positive reaction is usually obtained with most of the tuberculins.

4. Changes have been produced in animals which are indistinguishable from those found in Lymphadenoma; but much value is not placed on such experiments seeing that Baumgarten and Lichtenstein produced similar changes by the inoculation of small doses of ordinary tubercle bacilli.

Remarks. The authors' description of the histological lesions they found in some of their animals are not convincing. Also, in the light of my own animal experiments with acid-fast bacilli, I find it impossible to accept unreservedly the isolated experiments of the workers who claim to have produced typical Lymphadenoma in animals by the injection of small quantities of tubercle bacilli.

5. The body of the host weakens the bacillus, which is evidenced by: 1st, the fact that antiformin destroys the bacilli, while ordinary tubercle bacilli are not killed; 2nd, that the virulence can be heightened by the use of such a reagent as lactic acid; and 3rd, that on taking a series of animals, only certain members become tubercular.

Remarks. Here one may ask why the antiformin did not destroy the infective agent in the 1913 experiments. Also, the case 2 experiments seem to point to the possibility of the strength of the antiformin being rather weak. As regards the sensitising action of the lactic acid, cases 1, 2, 4 and 8 must obviously be left out of account. We then find that of the 15 animals inoculated with the gland alone, 4 developed disease and 11 remained healthy; of the 24 animals inoculated with the gland plus lactic acid, 11 became diseased and 13 remained healthy: thus 26·6 and 45·83 per cent. respectively of the animals inoculated became diseased. When the gland alone was used

there were only two cases of irregularity, where only some of the inoculated animals developed disease; with the lactic acid animals there were four cases of irregularity.

The animal experiments carried out in these laboratories, in an endeavour to confirm the results of the German workers, will now be briefly discussed.

Comparative animal experiments performed in these laboratories.

Specimens from 44 cases which were suspected clinically of being Lymphadenoma have been examined. On microscopical examination, 27 were found to be certainly lymphadenomatous, containing the typical karyokinetic giant cells and numerous eosinophile cells, etc.; three were probably lymphadenomatous, containing eosinophile cells, etc. but not the typical giant cells; seven showed a picture of chronic inflammatory changes, and were possibly lymphadenomatous; four were tubercular in nature; three were sarcomatous; one was later diagnosed as glandular fever, but the patient has since died of phthisis; and one which was thought to be an acute case of Lymphadenoma, only showed an acute periportal inflammatory condition of the liver. In three of the lymphadenomatous cases histological evidence of tubercular disease was also found, on the one hand the two diseases being intimately mixed with one another in several organs, while in one case lymphatic glands which appeared to be purely lymphadenomatous were associated with tubercle of the liver and lungs. On one or two occasions where ante-mortem specimens from a patient have been typical, post-mortem specimens from the same patient have been doubtful, and *vice versa*.

Of the 24 cases of Lymphadenoma, which were unaccompanied by tubercular disease, only 16 fresh specimens suitable for animal inoculations were obtained.

In Table II a list is given of the guinea-pigs inoculated with tissues obtained in the fresh state from the 16 certain cases of Lymphadenoma.

Cases 6, 14 and 29 showed a picture of chronic inflammation, case 16 of acute inflammation, case 19 was later diagnosed as glandular fever, and case 43 turned out to be a Lymphosarcoma. Case 34 showed histologically a picture of an old tubercular gland, with much caseation and calcification, the parasite having evidently been destroyed. None of the 94 animals became tubercular, and it will be noted that on 26 occasions the inoculum was treated with varying dilutions of lactic acid. Again, none of the 16 animals mentioned in Table III became tubercular, they being injected with material, probably lymphadenomatous; and the inoculum was treated with lactic acid on seven occasions. The 26 animals mentioned in Table IV also remained healthy, on nine occasions the inoculum being treated with lactic acid.

Thus we find that not one of the 136 animals inoculated with tissues obtained from suspected cases of Lymphadenoma developed tubercular disease, although on 42 occasions the inoculum was injected with lactic acid. It may be mentioned that a considerable number of animals have died at an early

date from diseases other than tuberculosis, all such animals being excluded from the above totals; only those which survived for at least six weeks after the injection being taken into account.

Table II. *Guinea-pigs inoculated with glands, etc., received in the fresh state, from typical cases of Lymphadenoma.*

Case	3.	2	guinea-pigs, with	fresh gland
		6	,, ,,	glycerinated gland
,,	7.	2	,, ,,	,, spleen
,,	8.	3	,, ,,	fresh gland
		6	,, ,,	gland kept in broth
,,	9.	3	,, ,,	,, saline
		3	,, ,,	,, broth
		7	,, ,,	glycerinated gland
,,	12.	2	,, ,,	fresh gland
,,	21.	2	,, ,,	,,
,,	23.	2	,, ,,	glycerinated gland
,,	24.	1	,, ,,	,, ,,
,,	26.	3	,, ,,	fresh gland
,,	27.	1	,, ,,	blood
		1	,, ,,	gland kept in broth
		1	,, ,,	bone marrow kept in broth
		1	,, ,,	gland plus lactic acid
,,	28.	2	,, ,,	gland kept in broth
		2	,, ,,	,, plus lactic acid
,,	30.	1	,, ,,	fresh gland
		3	,, ,,	gland plus lactic acid
,,	31.	1	,, ,,	,, kept in broth
		3	,, ,,	,, plus lactic acid
,,	36.	6	,, ,,	fresh gland
		6	,, ,,	gland plus lactic acid
,,	40.	6	,, ,,	fresh gland
		6	,, ,,	gland plus lactic acid
,,	41.	6	,, ,,	fresh gland
		6	,, ,,	gland plus lactic acid
Total		94		

Table III. *Guinea-pigs inoculated with gland, received in the fresh state, from cases probably lymphadenomatous.*

Case	22.	3	guinea-pigs, with	fresh gland
		1	,, ,,	gland kept in broth
		2	,, ,,	,, plus lactic acid
,,	42.	3	,, ,,	fresh gland
		3	,, ,,	gland plus lactic acid
,,	44.	2	,, ,,	fresh gland
		2	,, ,,	gland plus lactic acid
Total		16		

Table IV. *Material from cases diagnosed clinically as Lymphadenoma, but in which histologically there was no specific evidence of Lymphadenoma.*

Case	6.	6	guinea-pigs, with	gland kept in saline
,,	14.	1	,, ,,	fresh gland
		1	,, ,,	gland plus lactic acid
,,	16.	1	,, ,,	fresh gland
,,	19.	1	,, ,,	,,
,,	29.	1	,, ,,	,,
		2	,, ,,	gland plus lactic acid
,,	34.	6	,, ,,	fresh gland
		6	,, ,,	gland plus lactic acid
,,	43.	1	,, ,,	fresh gland
Total		26		

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It is very difficult to account for the striking difference between these figures and those obtained by Fraenkel and Much, not only in so far as the lactic acid experiments are concerned, but also as regards the controls. Among their 16 control animals which can with safety be considered, six developed disease, either ordinary tubercular disease or what the authors call a condition resembling Lymphadenoma. My 39 animals injected with untreated gland all remained healthy. Where lactic acid was used, 11 out of their 21 animals became diseased; again my 42 animals all remained healthy. As a matter of fact the 55 controls inoculated with material kept in saline, broth and glycerine are also of value for it has been found that tubercle bacilli kept in such liquids, in cold storage, survive for many months; the number of controls then becomes 94, all of which survived.

The injections made by Fraenkel and Much were with massive doses of the inoculum, and, in their opinion, this is one of the reasons why they were so successful in isolating the tubercle bacillus from such a number of cases. Many of my injections were, however, massive, and, what is more, many of them were repeated on several occasions. Frequently solid lumps of tissue were injected subcutaneously, which presumably would favour the development of any rare or fragile bacillus which might be present, but there was on no occasion resulting tubercular disease. The weights of my animals have been taken every week during the early stages of the experiments, in order, if possible, to register any transitory illness as a result of the injections. Provided the material inoculated has been free from ordinary bacterial contaminations, I have obtained no reliable evidence whatsoever that the injections affect materially the general health of the animals.

I can find no mention by Fraenkel and Much of control animal experiments with other lymphatic glands such as those obtained from cases of leucaemia or pernicious anaemia; it would be interesting to know what proportion of such diseased glands would, in their hands, infect the guinea-pig with tubercular disease.

In these laboratories the organs from about 20 cases of leucaemia and pernicious anaemia have been examined, and where there has been no histological evidence of tubercular disease, pieces of the tissues have been inoculated into animals. Some 50 guinea-pigs have been so inoculated, and six animals only developed tubercular disease among 12 inoculated with tissues derived from a single case of myelocytic leucaemia. None of the tissues were treated with lactic acid, for such control experiments, in these laboratories, were not considered necessary seeing that attempts to produce tubercular disease with lymphadenomatous glands failed.

Attempts were made to heighten the virulence of certain acid-fast bacilli by treating them with lymphadenomatous tissues and their extracts, and by simultaneous injection of the bacilli and the diseased tissues into animals. Saprophytic acid-fast bacilli were mostly used, and especially *B. phlei*. The last mentioned bacillus I consider to be only slightly less pathogenic for the

rabbit than the human tubercle bacillus, although with an equal area of diseased organs the wasting of the animal shows the greater toxicity of the tubercle bacillus. So far one has not been able to detect any special effect of lymphadenomatous tissues or their extracts on the virulence of the bacilli tested; and similar negative results were obtained when using carcinomatous and leucaemic tissues. It has been observed that the repeated injection of tubercular lymphadenomatous glands into the peritoneal cavity of guinea-pigs leads to death of the animals at a very early date, but one would imagine that glands from cases of other diseases secondarily infected with tubercle bacilli would similarly kill rapidly if injected repeatedly.

The lymphatic glands from a case of Lymphadenoma in which the liver and lungs were tubercular while the spleen and lymphatic glands appeared microscopically to be lymphadenomatous only, caused death of the six guinea-pigs inoculated within a few weeks. The histological picture in the organs of these animals was not that of typical tubercular disease, several massive injections of cell emulsions having been injected. The description given by Fraenkel and Much of the microscopical findings in some of their animals seems to correspond pretty well with that found in these six animals—giant cells containing several nuclei (not of Langhans' type) with fairly active mitosis and large areas of fibrosis practically free from cellular elements were present; but acid-fast bacilli were found, and the general appearance was not that of a lymphadenomatous organ.

It has been found that the presence of lymphadenomatous tissues or their extracts in fluid culture media has no noticeable effect on the rapidity or extent of the surface growth of tubercle bacilli and allied saprophytes.

CONCLUSIONS.

The evidence at present available in support of the view that Lymphadenoma is a special manifestation of tubercular disease is not conclusive: Lymphadenoma glands may often be infected with tubercle bacilli, but possibly not more often than they are infected with streptococci or diphtheroids.

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