ON THE TUBERCULIN SENSITIVITY OF EPITHELIAL CELLS *IN VITRO*

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(With Plates 14 and 15)

Necrosis following the injection of tuberculin into sensitized animals or found to be present in tuberculous lesions is commonly ascribed to the toxic effect of tuberculin on hypersensitive cells. Rich & Lewis (1932) have submitted evidence of such an effect in tissue culture. The cells studied by these and most other investigators were fibroblasts and various types of leucocytes. Observations on non-mesenchymal elements have been few. Buckley, Buckley & Keeve (1951) have claimed that tuberculin inhibits the growth in tissue culture of the liver cells of sensitized rabbits, but Cruickshank (1951), working with guinea-pigs, could not detect any adverse effect on the growth of the epidermal cells in small pieces of whole skin cultured in a fluid medium containing antigen. In the present work the outgrowth of epithelium in tissue culture from liver and kidney explants derived from sensitized guinea-pigs has been studied. Clear evidence was obtained of the indifference to tuberculin of such epithelium.

EXPERIMENTAL METHODS

The methods used to prepare a purified form of tuberculin and to sensitize guinea-pigs to tuberculin with B.C.G. have been described previously (Marks, 1953). Animals prepared in this manner were used as donors of sensitized tissue, as were also others infected with virulent tubercle bacilli. The latter tuberculous animals were used 5–6 weeks after infection when they showed disseminated lesions. The preparations of tuberculin used, which were free of antiseptic, were equivalent in potency to standard Old Tuberculin. In some of the preliminary experiments a purified tuberculin, I.P. 48, was employed, kindly provided by Dr J. Bretey of the Pasteur Institute.

Media

In the first series of experiments the tissue culture medium, A, was composed of equal amounts of two solutions. One contained guinea-pig serum, horse serum, chick embryo extract, Tyrode solution and streptomycin solution in equal proportions; the other was a mixture of cock plasma and an equal volume of either purified tuberculin solution or, in control cultures, Tyrode solution. The final concentration of streptomycin in medium A was 1 unit/ml. Penicillin (10–20 units/ml.) was also included when the tuberculin I.P. 48, which had not been prepared aseptically, was employed.

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To provide plasma for the medium B used in the second series of experiments, 15 ml. of hen blood were collected into 10 ml. of Tyrode solution containing a total of 25 units of heparin, 800 units of penicillin and 80 units of streptomycin. For setting up cultures the plasma solution obtained by centrifugation was clotted with an equal volume of a mixture of chick embryo extract 1·3 ml., horse serum 0·25 ml., guinea-pig serum 0·25 ml. and tuberculin or Tyrode solution 0·2 ml. Medium B proved very favourable for the growth of kidney epithelium but not for liver explants, which appeared to impair the consistency of the plasma clot.

Medium C was composed of heparinized guinea-pig plasma clotted with half its volume of an extract of guinea-pig spleen. It was used primarily for studies on macrophages and fibroblasts, to be reported separately, but observations on epithelial activity were made at the same time. Medium C proved less satisfactory than A for liver epithelium, possibly owing to the density of the plasma clot, the outgrowths in it being small and degenerating quickly. It was quite unsuitable for kidney explants, which caused rapid and widespread liquefaction of the clot.

The technique of tissue culture used in the present work was the hanging-drop method in slide cells. In most instances three explants were placed in each hanging drop. In some experiments the pieces were implanted near or on the surface of the coagulum. It appeared that with liver some better epithelial membranes were obtained in this way, but not so with kidney. In fact, the outgrowth of epithelium from kidney explants was often particularly luxuriant when they had been embedded in the clot. Preparations in medium A were observed during 5–6 days' incubation at 37° C. and those in the other media during 7 days. Throughout this paper, the concentrations of tuberculin described are the final concentrations in the completed medium.

RESULTS

Guinea-pig kidney

Epithelial outgrowths, often of considerable size, were observed in a large proportion of tissue cultures, especially in medium B. Purified tuberculin had no adverse effect on the incidence of epithelial outgrowths, the rate of their spread or final extent. In the presence of a final concentration of tuberculin of 1/20 in medium B, 132 of 179 explants produced epithelial outgrowths (74 %), and in the absence of tuberculin, 101 of 129 (78 %). The explants were derived from nine tuberculous and two B.C.G. animals, each animal providing approximately sixteen test and twelve control cultures. Epithelial outgrowths in medium B were very transparent and not well suited for photography, an effect possibly connected with the expression of fluid from the medium during incubation. In three cultures in medium B containing 1/20 tuberculin, each of which showed large epithelial outgrowths, the persistence of active tuberculin after 3, 5 and 7 days' incubation respectively was demonstrated by injecting intradermally into tuberculous and normal guinea-pigs 0·1 ml. volumes of a 1/10 dilution of the fluid expressed by the medium. Strongly positive tuberculin reactions were obtained in the tuberculous animals and negative reactions in the normals.

The tuberculin concentrations employed in medium A were varied from 1/20 to 1/60 without effect on the incidence of epithelial outgrowths, which in the combined group amounted to 49 out of 114 explants (43 %) compared with 17 out of 60 (28 %) in the same medium without tuberculin. Control and test explants were obtained from three guinea-pigs sensitized with B.C.G. The corresponding results for explants from two normal animals in medium A were: 49 explants active out of 64 in the presence of tuberculin (77 %) and 27 out of 45 in its absence (60 %). In Pl. 14, fig. 1, is shown an outgrowth of kidney epithelium cultivated for 5 days in medium A containing 1/60 tuberculin from the tissue of a guinea-pig sensitized with B.C.G.

Guinea-pig liver

The incidence of epithelial outgrowths from liver explants was low. The macrophage and fibroblast activity usually seen in these liver cultures will not be considered here. In medium A, 15 of 174 explants derived from six B.C.G. animals exhibited epithelial outgrowths in the presence of tuberculin concentrations of 1/20 to 1/60 (8·6 %) and 5 of 103 explants in the absence of tuberculin (4·8 %). The corresponding results for liver explants from four normal animals cultured in medium A were: 6 explants active out of 109 in the presence of tuberculin (5·5 %) and 2 out of 79 in its absence (2·5 %). Pl. 14, fig. 2, shows the epithelial outgrowth from a liver explant cultivated for 6 days in medium A containing 1/60 tuberculin, the donor guinea-pig having been sensitized with B.C.G.

In medium C, containing 1/20 tuberculin, epithelial outgrowths were observed in 8 of 114 liver explants, which were obtained from eleven tuberculous animals (7·0 %) (Pl. 14, fig. 3). In the same medium without tuberculin, 7 of 128 explants from the same donors showed similar outgrowths (5·5 %).

The observations made on cultures of tissue obtained from sensitized animals are summarized in the Table.

Table 1. *The incidence of epithelial outgrowth in tissue culture of liver and kidney explants from sensitized guinea-pigs in the presence or absence of tuberculin*

Medium	Tissue	Type of bacilli used to sensitize	No. of donor animals	Concentration of tuberculin	No. of explants	No. showing epithelial growth	%
A	Kidney	B.C.G.	3	1/20–60	114	49	43
				Nil	60	17	28
B	Kidney	Virulent B.C.G.	9, 2	1/20	179	132	74
				Nil	129	101	78
A	Liver	B.C.G.	6	1/20–60	174	15	8·6
				Nil	103	5	4·8
C	Liver	Virulent	11	1/20	114	8	7·0
				Nil	128	7	5·5

DISCUSSION

The experimental results described above agree with and amplify those obtained by Cruickshank with skin explants (1951), but fail to corroborate those of Buckley *et al.* (1951). Our kidney cultures from sensitized animals show, without any doubt, the complete indifference of the growing epithelium to the presence of tuberculin

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in the medium. The results with liver explants, though similar in nature, weigh less heavily because of the generally low incidence of epithelial outgrowth. This sporadic occurrence of epithelial growth from adult liver in such plasma-clot hanging-drop cultures is well recognized, although a slightly better yield of epithelial outgrowth can be obtained from explants of regenerating liver for a short period after partial hepatectomy (Abercrombie & Harkness, 1951). In contrast, Buckley, Buckley & Gey reported in 1949 having obtained outgrowth of parenchymal liver cells from adult rabbits in as high a proportion as 90% of their cultures. However, their illustrations, showing mostly rather elongated cells with predominantly elongated oval nuclei and with frequent spaces, often of considerable size, separating the cells, do not clearly identify the outgrowths as epithelial. In the paper by Buckley *et al.* (1951), which is of immediate concern, the frequency of epithelial growth obtained is not given. In the illustrations accompanying this paper there are, besides elongated separated cells,* also some more compact cell groups.

In the present work all the epithelial outgrowths observed were composed of sheets of contiguous cells. However, in order to obtain additional evidence of the epithelial nature of these sheets, some representative cultures were, after a brief wash in sodium sulphate, directly impregnated with silver nitrate followed by development in amidol dissolved in sodium sulphite. In this way, the mosaic pattern of the cell outlines typical for such epithelial membranes was clearly demonstrated (Pl. 15, figs. 4, 5).

No attempt was made to determine whether the epithelial outgrowths from liver explants were derived from the parenchymal cells or from bile duct epithelium.

The results of the present work suggest that tuberculin has no *general* cytotoxic effect *in vivo* in sensitized animals. Its effect on certain types of mesenchymal cell, although the subject of conflicting reports, appears to have been more definitely established by previous workers, but further investigations seem desirable in order to elucidate the conditions necessary for its demonstration.

SUMMARY

Purified tuberculin in concentrations equivalent to 1/20 standard Old Tuberculin had no apparent effect on the outgrowth *in vitro* of liver and kidney epithelium derived from sensitized guinea-pigs.

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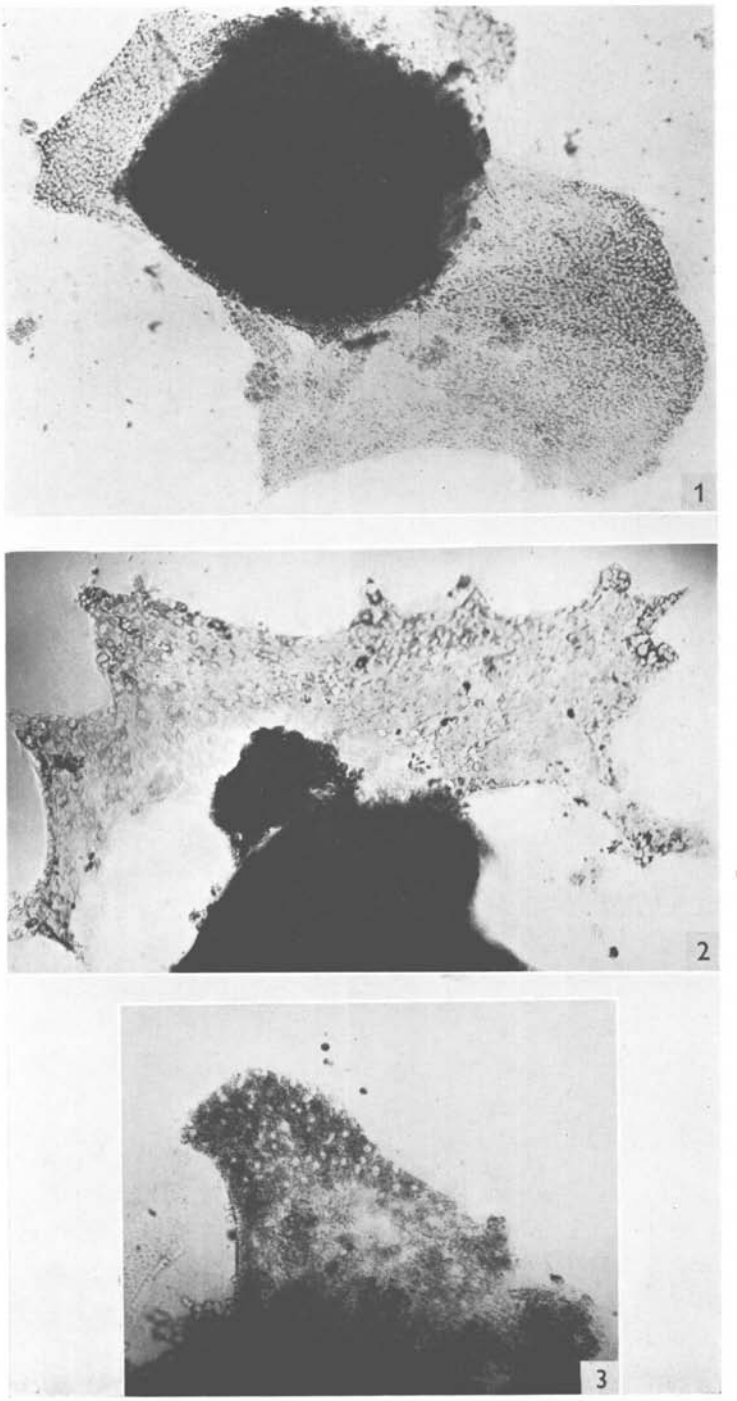
* In a personal communication Dr J. J. Buckley informs us that besides these cells fibroblasts and macrophages were also observed.



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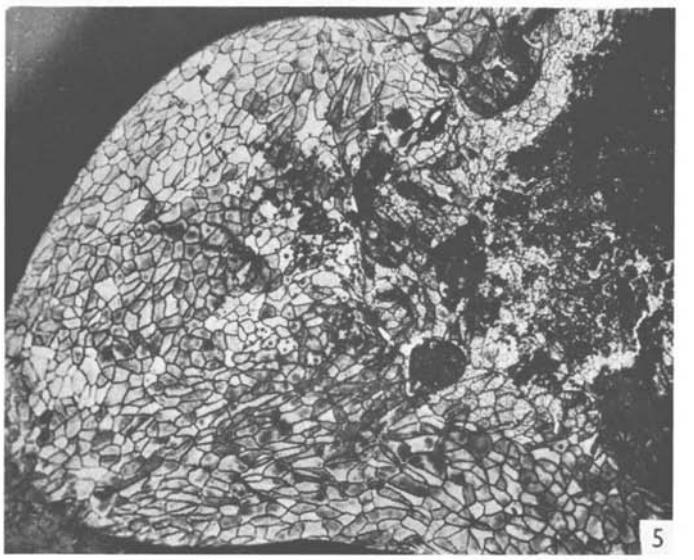

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EXPLANATION OF PLATES

PLATE 14

Fig. 1. Epithelial membranes growing from kidney explant of a sensitized guinea-pig in medium A containing purified tuberculin 1/60. Photograph of the living hanging-drop culture 5 days after explantation. × 50.

Fig. 2. Epithelial membrane growing from liver explant of a sensitized guinea-pig in medium A containing purified tuberculin 1/60. Photograph of the living culture 6 days after explantation. × 66.

Fig. 3. Epithelial membrane growing from liver explant of a sensitized guinea-pig in medium C containing purified tuberculin 1/20. Photograph of the living culture 4 days after explantation. Approx. × 80.

PLATE 15

Fig. 4. Part of same culture as in fig. 1, 2 days later and after direct silver impregnation, to show the mosaic pattern of cell outlines typical for the surface interfaces of epithelial cell communities. The field is the mirror image of the membrane on the right in fig. 1, the culture now being mounted in canada balsam and photographed from above. × 48.

Fig. 5. Epithelial membrane of kidney explant from a normal guinea-pig; culture grown in medium A containing purified tuberculin 1/40, 7 days after explantation. Direct silver impregnation. Note widely and well displayed mosaic pattern. × 84.

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ERRATUM

In the paper by Marks, J. and James, Dinah M. entitled 'The effect of tuberculin on sensitive and normal leucocytes', *J. Hyg.*, 1953, vol. 51, p. 344, the sentence commencing 'These observations are consistent...' should have been deleted.