TISSUE CULTURE STUDIES ON BACTERIAL HYPERSENSITIVITY

I. Tuberculin Sensitive Tissues

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Plates 22 and 23

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Tissue culture methods have been but little used in the elucidation of the various problems encountered in bacterial hypersensitivity. The few studies reported have dealt with tissues from tuberculin sensitive animals. Rich and Lewis (1) showed that tuberculin in proper concentration had a selective toxic effect on cells from tuberculous animals. They believed that the tuberculin sensitivity was inherent in the cells, and that the cytotoxic effect was the result of an antigen-antibody reaction. Aronson (2) confirmed this work and also demonstrated the cytotoxic specificity of various tuberculins on sensitive cells as compared with an indifferent effect of extracts made from other acid-fast organisms. In another series of experiments he (3) compared the reaction of tissues from animals sensitized to proteins with that of tissues sensitive to tuberculin when the respective antigens were added to the culture media. Horse serum added to cultures of tissues from horse serum sensitized animals produced no demonstrable cytotoxic effect; this was in marked contrast to the cytotoxic action of tuberculin on tissues from tuberculous animals; he thus showed that there is a fundamental difference between the two types of hypersensitive tissues. We (4) have also noted that cells from animals sensitized with horse serum, egg albumin or beef lens were not specifically inhibited when the respective antigens were added to tissue cultures. As a preliminary to an analysis of other types of bacterial hypersensitive states it was thought advisable to make a detailed study of tuberculin allergy since this is a prototype. This communication deals, therefore, with a partial repetition of previous work with amplifications and extensions.

EXPERIMENTAL

Animals.—The animals were young rabbits weighing from 1,000 to 1,300 gm., and male albino guinea pigs weighing from 300 to 400 gm.

Tubercle Bacilli.[1]—Three strains of tubercle bacilli were used: B 1, a bovine strain virulent for rabbits; H 37, a human strain, virulent for guinea pigs; and R 1, a human strain of very low virulence even for guinea pigs. The bacilli were grown on plates of Corper's egg medium, harvested, weighed and suspended in normal saline by grinding in a mortar so that 0.2 cc. of the suspension contained the desired amount of organisms for each animal inoculation. Rabbits were inoculated with 0.1 mg. of strain B 1 intravenously, and guinea pigs with 0.1 mg. of strain H 37 or 5.0 mg. of strain R 1, subcutaneously or intratesticularly.

Tissue Culture Media.—Carrel micro flasks and homologous media were used throughout; *i.e.* normal rabbit plasma with 25 per cent rabbit embryo extract, or 10 per cent rabbit splenic extract for growth of rabbit tissue; and normal guinea pig plasma with 10 per cent guinea pig splenic extract were employed for guinea pig tissues. 4 cc. of blood obtained by cardiac puncture was placed in each chilled tube containing 0.5 cc. of heparin solution (concentration for rabbit blood was 1–1,000 and for guinea pig blood was 1–700 in Ringer's solution[2]). The tubes were kept cold, centrifuged at high speed, after which the plasma was removed and pooled. Tissue extracts were prepared by finely mincing embryonic or splenic tissue and adding Tyrode's solution to make the required strength. The suspension was thoroughly mixed, allowed to stand for ½ hour, then centrifuged at high speed for a similar period. The clear, slightly opalescent supernatant fluid was used as tissue extract. 1 cc. of normal plasma and 0.5 cc. of tissue extract were used in each flask and mixed just before transferring the explants.

Tuberculin Concentration.—Tuberculin[3] suitably diluted in Tyrode's solution was mixed with plasma so that it comprised one-tenth of the total volume of the media in the flask. The final dilution of tuberculin used in the media was from 1–200 to 1–300, as this concentration had but slight inhibitory effect on normal cells.

Explants.—Splenic and testicular explants from both rabbits and guinea pigs

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[1] The authors wish to acknowledge the receipt of various strains of tubercle bacilli from the following: Dr. Florence R. Sabin for the H 37 and B 1 strains; Dr. S. A. Petroff, of Trudeau Sanatarium, and Miss Lucy Mishulow, of the City of New York Bureau of Laboratories, for R 1 strains.

[2] A more highly purified heparin has been obtained from the Connaught Laboratories, Toronto, Canada, and this has been used in later experiments. The most suitable concentration for guinea pig blood was found to be about 0.5 cc. of a 1 to 8,000 solution for 4 cc. of blood.

[3] Tuberculin and glycerin broth controls were kindly supplied by Dr. John Reichel, Director of Mulford Laboratories, Sharp and Dohme. The same lot of tuberculin was used throughout these experiments.

were used. Splenic explants, especially from the guinea pig, contained an abundance of wandering cells and fibroblastic elements; they therefore were most satisfactory for our studies, and were most often employed. The animals were killed by a sharp blow over the head; the tissues were removed aseptically; the central portions were cut into explants approximately 1 mm. square and were washed in Tyrode's solution. Four explants were transferred to each flask as soon as the media were thoroughly mixed. Twelve explants were used for each experimental condition. Incubation was carried out at 37.5°C.

Experimental Observations.—Qualitative and quantitative estimations of the effect of tuberculin on sensitive and normal cells were made daily. Microscopic examinations, revealing changes in the size, shape, color and amount of granulation of the cells, indicated the different degrees of toxicity of the tuberculin. Quantitative estimations of the relative increase in areas of wandering cell migration and of fibroblastic growth were determined by ocular micrometric (5) and by projectoscopic methods (6) respectively. The areas of cellular migration were roughly circular; and since the area of a circle is directly proportional to the square of its radius, the square of the average radius of the twelve explants and growths in each experimental set up were compared. Fibroblastic growths were more irregular in outline so that their areas were determined by projectoscopic methods.

Definition of Quantitative Terms Used.—

$$\text{Rate of migration or growth} = \frac{\text{Area of growth} - \text{area of explant}}{\text{Area of explant}} \text{ per unit of time}$$

$$\text{Cytotoxic index} = \frac{\text{Rate of growth in media containing tuberculin}}{\text{Rate of growth in media not containing tuberculin}}$$

If the cytotoxic index is approximately 1, the tuberculin in that concentration has an indifferent effect; if distinctly less than 1, it is toxic.

$$\text{Comparative cytotoxic index} = \frac{\text{Cytotoxic index of tuberculin on test explants}}{\text{Cytotoxic index of tuberculin on normal explants}}$$

If the comparative cytotoxic index is about 1, the test tissue is not sensitive to tuberculin; if definitely less than 1, the test tissue is sensitive or specifically inhibited; in other words, tuberculin has a specific cytotoxic effect on the sensitized tissue.

The initial growth energy refers to the growth rate of tissue in normal media.

$$\text{Comparative initial growth index} = \frac{\text{Rate of growth of test explants in normal media}}{\text{Rate of growth of normal explants in normal media}}$$

RESULTS

Course of the Experimental Tuberculosis.—The bovine strain, B 1 of tubercle bacilli, induced in rabbits a slowly progressive disease involving many viscera, with cachexia and a lethal outcome.

The H 37 virulent human strain injected subcutaneously into guinea pigs induced a local caseous lesion which usually drained and healed. Progressive involvement of the regional lymph nodes was followed by other visceral disease, and the infection usually terminated fatally after a period of weeks or months. Moderate to marked splenomegaly usually occurred.

The R 1 human strain of low virulence, usually injected intratesticularly, caused a local inflammatory reaction with only moderate general reaction. When this strain was injected subcutaneously, regional lymphadenopathy as well as a local abscess developed. During the acute stage multiple white punctate hepatic lesions and slight to moderate splenomegaly occurred. After the acute local inflammation subsided, the splenomegaly decreased; the animals gained weight and appeared generally healthy. A fatal outcome seldom occurred. The local lesion in the testicle persisted for months as a caseous mass in which acid-fast bacilli in large numbers could be demonstrated.

Tuberculin Reactions in Guinea Pigs.—Positive reactions of the delayed inflammatory type were elicited when 1.0 mg. of human old tuberculin was injected intracutaneously. A maximal reaction was usually reached at 24 hours. Similar skin responses to tuberculin were elicited in animals infected with both strains R 1 and H 37. Seriously ill or moribund pigs usually gave hypoergic reactions.

Histologic Picture of Organs from Which Explants Were Obtained.—Sections of spleens from animals infected with the virulent H 37 strain showed extensive epithelioid cell hyperplasia, especially of the Malpighian bodies, with numerous giant cells. Typical tubercles with areas of central necrosis were frequently found.

Splenic sections from animals infected with strain R 1 showed marked epithelioid cell hyperplasia with numerous giant cells during the early acute toxic phase in which splenomegaly was present. Areas of central necrosis were not noted. The histologic picture during the healing stage, in which the spleen returned to normal size, showed regression of the lesions to a nearly normal picture.

Tissue Culture Observations

Cellular Migration and Fibroblastic Growths from Explants in Normal Media.—Migration of small wandering cells, mostly polymorphonuclear, appeared soon

after explantation of splenic tissue. These cells continued to wander out into the medium away from the explant for about 24 hours, when they began to degenerate and disintegrate. By the 2nd day numerous large wandering cells of the macrophage type were in evidence. With each succeeding day the cells became larger, the protoplasmic processes more complex and the extent of migration greater. The advancing line of cells was usually sharp and the outline roughly circular. After 4 days the cellular migration from explants in normal plasma had usually extended so far that cells from different explants in the same flask intermingled; this rendered further quantitative measurements impossible. Fibroblasts from splenic explants appeared 2 or 3 days after explantation and grew out as solid sheets of cells. Testicular explants produced almost entirely fibroblastic forms with but few scattered wandering cells.

Qualitative Effect of Old Tuberculin on Cells from Normal and Tuberculous Animals.—Preliminary experiments showed that tuberculin in a concentration of 1–300 had but slight effect on cells from normal animals; this visible effect was chiefly a slight increase in the amount of granulation, attenuation of protoplasmic processes, or a slight decrease in cell size. Most of the normal cells in the presence of this concentration of tuberculin appeared healthy and active at the termination of experimental observations.

Cells from tuberculous animals, on the other hand, were severely injured by the tuberculin.

The more highly sensitive cells migrated only short distances before they died, degenerated and disintegrated. Cells with lesser degrees of sensitivity survived longer and migrated further. Various grades of coarse granulation and vacuolization of the cytoplasm developed; protoplasmic processes shortened; the cells became smaller and rounder; and cellular disorganization was followed by disintegration. The cytotoxic effect of tuberculin on fibroblasts from tuberculous animals was roughly parallel, but as a rule these cells were somewhat more resistant. Figs. 1 to 8 show the comparative effect of tuberculin on sensitive and normal macrophages and fibroblasts. Tuberculin had a similar cytotoxic effect on fibroblastic growth from testicular explants.

Quantitative Determinations of Cellular Migration and Fibroblastic Growth.—The extent of splenic cellular migration was measured daily by means of ocular micrometric methods, and the amount of testicular fibroblastic growth by projectoscopic and planimetric determinations. The measurements of cellular migration from the twelve explants within each experimental condition usually varied but little, so that

the average obtained was a valid figure which represented quantitatively the effect of the tuberculin. Comparative cytotoxic indices of the effect of the old tuberculin on cells from tuberculous and normal animals, shown in Chart 1, were always well below 1, indicating specific cytotoxicity of tuberculin on cells from tuberculous animals. In this chart the comparative cytotoxic index for each of 20 experiments is represented by a suitable sign; this index is plotted against the duration of the infection. The comparative indices represented

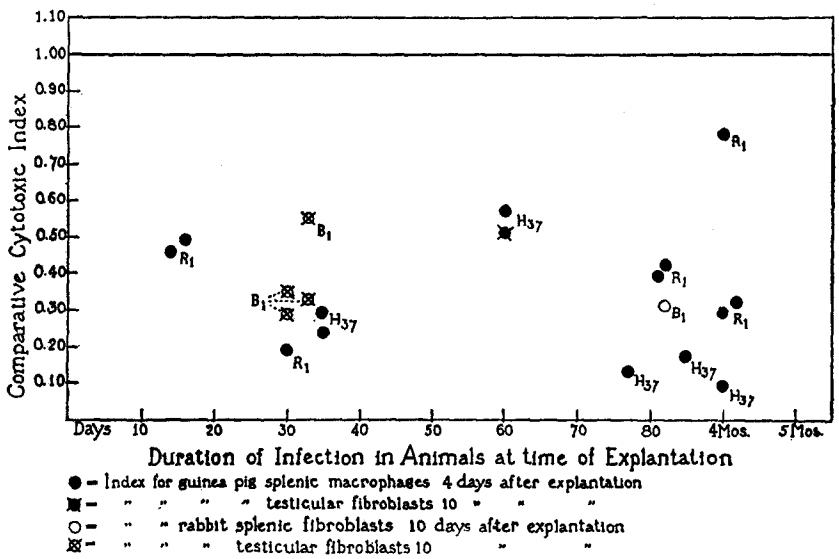

CHART 1. Comparative cytotoxic indices of tuberculin for macrophage migration or fibroblastic growth for explants from tuberculous guinea pigs and rabbits. The small figure near each index indicates the infecting strain of tubercle bacillus.

were determined 4 days after explantation in the case of splenic wandering cells and 10 days after explantation in the case of testicular fibroblasts. These indices varied from 0.09 to 0.78. Tissues from tuberculous animals were found sensitive as early as 14 days after inoculation. Splenic and testicular fibroblasts were about equally sensitive. Cells from animals infected either with the highly virulent H 37 or with the lowly virulent R 1 strains showed similar degrees of sensitivity to the tuberculin. This marked sensitivity persisted in

cells from R 1 infected guinea pigs at least as long as 4 months after infection, at which time the spleens were essentially normal macroscopically and microscopically; and the only demonstrable foci of infection were small caseous abscesses at the original sites of infection.

Correlation between the Microscopic Appearances and Quantitative Migration and Growth of Cells.—As a rule there was a close correlation between the microscopic appearances of cells and the comparative indices of tuberculin cytotoxicity.

Markedly sensitive cells migrated only slightly in media containing tuberculin and were soon killed; hence comparative indices were correspondingly low. Lesser degrees of sensitivity were manifested by greater activity of the cells and higher indices. A prime requisite for assuring validity of quantitative measurements is the production of firm fibrin clots in which the explants were placed. Firmer clots were obtained when the plasma was but slightly diluted. In instances where excessive fluid collects over the explant some of the surface cells float beyond the margin of cellular migration. With a little experience these cells can be easily recognized and thereby false figures for cellular migration avoided.

Specificity of Tuberculin Toxicity on Sensitive Cells.—In order to test the specific toxicity of old tuberculin on sensitive tissues, other cytotoxic materials such as concentrated glycerin broth, autolysates of various streptococci, streptococcal extracts and proteins were used. Suitable dilution of these substances having but slight effect on normal cells, had little, if any, greater effect on tuberculin sensitive cells, thereby indicating the specific toxicity of tuberculin.

Duration in Vitro of Cellular Sensitivity to Tuberculin.—In view of the persistence of cellular sensitivity to tuberculin months after the acute local inflammatory reaction had subsided in animals infected with strain R 1, it seemed advisable to determine the duration of this sensitivity *in vitro* when cells from tuberculous animals were grown in media containing normal plasma and extract.

Several experiments were undertaken with splenic explants from guinea pigs, 1, 3 and 4 months respectively after intratesticular infection with strain R 1. The two animals infected 3 and 4 months previously were in good general condition, both reacted positively to tuberculin injected intracutaneously, and at autopsy both showed only small caseous tuberculous abscesses in the inoculated testicle. No other macroscopic evidence of tuberculosis was discernible; and, except for slight enlargement of the spleen in one animal, no other abnormality was noted. Smears from the testicular foci showed numerous acid-fast bacilli. Culture of

finely minced splenic tissue on Corper's egg medium failed to grow tubercle bacilli. Sections of spleen failed to show acid-fast bacilli.

In Experiment 212 the comparative cytotoxic index was 0.29, 4 days after explantation, as shown in Table I, indicating that the cells from the tuberculous animal were markedly sensitive to tuberculin. Fibroblastic growths from the tuberculin sensitive and normal explants, grown in normal plasma without tuberculin, were allowed to proliferate for 10 days, at which time large sheets of cells had formed. Transplants were made from these growths in the usual manner, by selecting actively growing cells toward the periphery and discarding the central portions. These transplants were then placed in new media so that half of each of the sensitive and normal transplants were placed in media containing the same

TABLE I

Persistence in Vitro of Cellular Sensitivity to Tuberculin

Experiment No.	Duration of tuberculous infection (strain R1)	Comparative cytotoxic indices of tuberculin after explantation		Comparative cytotoxic indices after first transplantation of fibroblasts		Comparative cytotoxic indices after second transplantation of fibroblasts	
		Day	Index	Day	Index	Day	Index
	mos.						
212	4	4th	0.29	5th (15)	0.25		
				7th (17)	0.28		
				9th (19)	0.29		
218	3	4th	0.42	4th (15)	0.30		
				7th (18)	0.28	4th (25)	0.56
				9th (19)	0.30	7th (28)	0.41
242	1	4th	0.37	4th (12)	0.32	4th (20)	0.51
				6th (14)	0.41	6th (22)	0.72

The numbers in parentheses after the days of transplantation indicate the number of days since the original explantation.

concentration of tuberculin as in the original set up, and the other half of similar transplants were placed in control media without tuberculin. Comparative cytotoxic indices of fibroblastic growth determined on the 5th, 7th and 9th day after transplantation, or, in other words, on the 15th, 17th and 19th day after the original explantation, were 0.25, 0.28 and 0.29 respectively; this showed that tuberculin sensitive cells maintained their sensitivity after proliferation in normal media in tissue culture. The few scattered macrophages carried over with the fibroblastic growths also exhibited sensitivity to tuberculin.

In a similar experiment (218, Table I) explants from another animal, infected with strain R 1 for 3 months, were specifically inhibited and had a comparative index of 0.42. Transplantation of fibroblastic growths was made as before with

resulting comparative indices of 0.30, 0.28 and 0.30 on the 4th, 7th and 9th day respectively. Secondary transplants had comparative indices of 0.56 and 0.41,

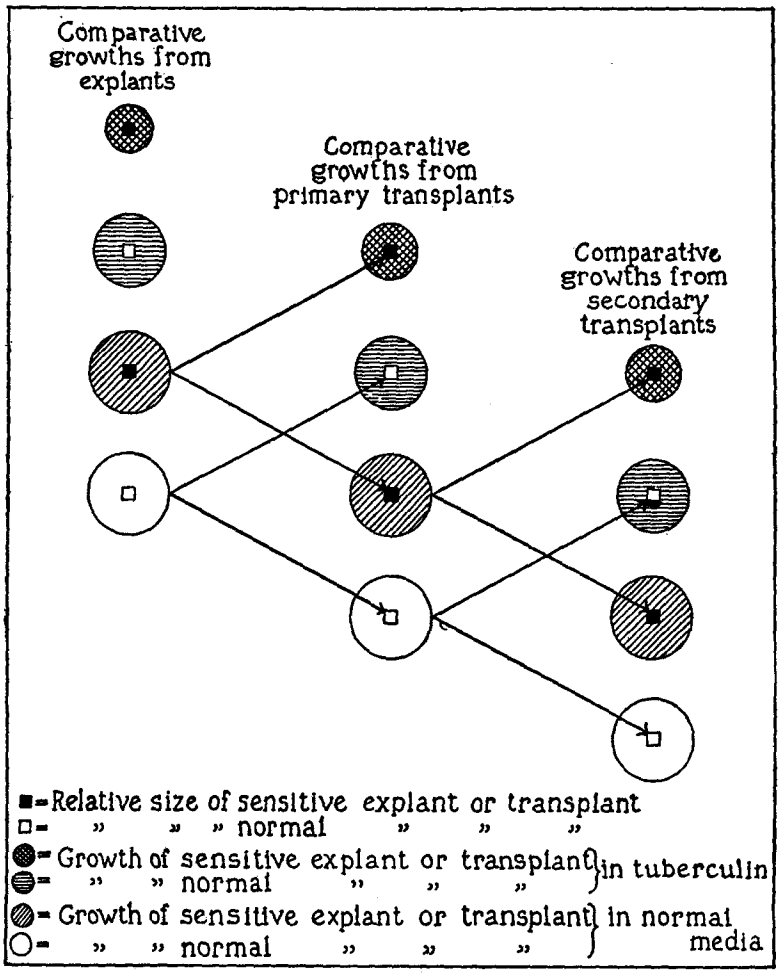

CHART 2. Diagrammatic representation of persistence *in vitro* of cellular sensitivity to tuberculin. The arrows indicate the division of fibroblastic growths into transplants.

on the 4th and 7th day after transplantation, or the 25th and 28th day after explantation, respectively; this indicated that tuberculin sensitive cells still maintained their sensitivity after two transplantations during which time many genera

tions of cells must have resulted by proliferation in artificial culture. The course of events in Experiment 218 is graphically represented in Chart 2.

In Experiment 242 (Table I) the animal had been infected with strain R 1 for 1 month. Culture of the minced spleen on Corper's media failed to show growth of tubercle bacilli. A heavy suspension of the minced spleen injected subcutaneously into a normal guinea pig produced no local abscess or adenitis, and autopsy 1 month later showed no macroscopic evidences of tuberculous infection. The comparative cytotoxic index 4 days after explantation was 0.37. On the 4th and 6th day after primary transplantation the comparative indices were 0.32 and 0.41 respectively. On the 4th and 6th day after secondary transplantation, that is, on the 20th and 22nd day after original explantation, the indices were 0.51 and 0.72 respectively.

Experiments 218 and 242 show that there is a very gradual loss of cellular sensitivity to tuberculin on prolonged growth in normal media. In Experiment 242 several of the explant fibroblastic growths in normal media were excised, sectioned and stained for acid-fast bacilli, but careful search failed to reveal their presence. It thus appears in the above experiments that tubercle bacilli were absent or if present were undetectable in the explanted spleens from strain R 1 infected animals, since it has been impossible to demonstrate acid-fast bacilli by culture, by animal inoculation or by stained sections of fibroblastic growths from the explant. It therefore seems improbable that growth of tubercle bacilli in the explanted tissue is the explanation for the persistence of cellular sensitivity to tuberculin *in vitro*.

Comparison of Initial Growth Energy of Tuberculin Sensitive and Normal Explants.—In view of the varying pathological picture of sections of spleens from which explants were taken it seemed probable that there would be a difference in the initial growth energy of the tuberculin sensitive cells, depending on the stage of infection and type of infecting tubercle bacillus. In Chart 3, which shows the comparative initial growth indices of thirteen experiments using guinea pig splenic explants, it is seen that cells from strain H 37 infected animals were less active, with indices varying from 0.28 to 0.53. The activity of tuberculin sensitive cells from animals infected with strain R 1 was moderately retarded early in the course of the infection (indices 0.49 to 0.72) during which time a splenitis was demonstrable. 3 and 4 months after infection when the spleens were practically normal macroscopically and histologically, the growth indices were nearly normal and varied from 0.83 to 1.18. There was thus a close cor-

relation between the pathologic picture of the spleens and the growth capacity of splenic explants in normal media. Explants from spleens exhibiting marked pathological features produced much less vigorous growths than did explants from spleens nearly normal histologically.

Correlation between Initial Growth Energy and Tuberculin Sensitivity of Splenic Explants.—By comparing Charts 1 and 3 it is seen that there is no distinct correlation in this respect. Splenic explants from animals late in the course of the infection with strain R 1, at which time the spleens were nearly normal histologically, were almost as

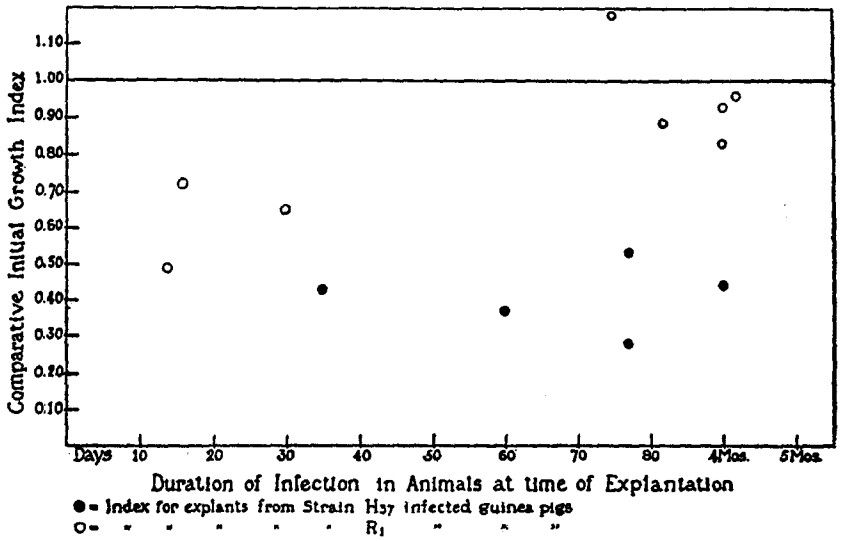

CHART 3. Comparative initial growth (macrophage migration) indices for splenic explants from tuberculous guinea pigs 4 days after explantation.

sensitive to tuberculin as were explants from spleens, pathological in the gross, of guinea pigs infected with strain H 37.

DISCUSSION

These experiments clearly show that human and bovine old tuberculin have a marked specific toxic effect on explanted cells from animals infected with various strains of tubercle bacilli, and corroborate the work of previous investigators (1, 2). There appears to be no definite correlation between the virulence of the infecting tuber-

cle bacillus or the extent of tuberculous lesions as regards the resulting sensitivity of cells to tuberculin. Cells from animals infected with lowly virulent strain R 1 are nearly as much inhibited by tuberculin as are cells from animals infected with a virulent strain, H 37, in which extensive progressive visceral lesions occur.

The specificity of the cytotoxic effect of tuberculin on sensitive cells has been established by showing that certain other toxic bacterial products do not exhibit this selective inhibition on cells from tuberculous animals. Aronson (2) has also shown that there is a certain degree of specificity of extracts prepared from various acid-fast organisms in regard to their cytotoxic action.

The inherent sensitivity of tuberculin sensitive cells has persisted for at least two transplantations *in vitro*, during which time the original cells proliferated to form many new generations. The original explants from animals infected with strain R 1 were apparently free from living tubercle bacilli since cultures and stained sections of, and animal inoculations with, similar portions of the spleens failed to reveal the presence of these microorganisms. These cells, although grown *in vitro* in a medium free from the products of tuberculous infection, other than those present in the explants themselves, continued to possess this tuberculin sensitive characteristic through repeated generations. There was, however, a gradual decrease in the degree of sensitivity on prolonged culture.

If the sensitivity is inherent in the cell, as available evidence indicates, this can be more clearly demonstrated by using smaller aggregates or single cell cultures. Although explants measuring only 1 mm. in diameter are comparatively small, still this mass of tissue is composed of thousands of cells, and complete removal of body fluids by washing in Tyrode's solution seems improbable, except possibly by perfusion. These body fluids carried over with the explants may diffuse into the media as the explants grow, and may possibly play a rôle in sensitizing the newly grown cells. Experiments are in progress to analyze further these problems and also to attempt sensitization of normal cells to tuberculin *in vitro*.

The nature of the specific cytotoxic effect of tuberculin on cells from tuberculous animals also requires further elucidation. Rich and Lewis (1) suggest that it is an antigen-antibody type of reaction.

On the other hand, there is a possibility that the tuberculin cytotoxicity is the result of an additive effect. In this case one must assume that some toxic material similar to tuberculin bathes the cells in tuberculous animals, and when these tissues are explanted into culture media containing tuberculin, a combined action of the tuberculin and this hypothetical toxic substance results. This explanation seems very improbable, however, as specific tuberculin sensitivity can be demonstrated over a wide range of tuberculin concentrations.

The toxic effect of tuberculous infection on body cells, particularly of the wandering or macrophage type, was manifest *in vitro* by decreased activity. Cells from an animal infected with a lowly virulent strain R 1 of tubercle bacillus showed, during the toxic stage when grown in normal media, a decrease in migratory ability of macrophages and a decrease in quantitative fibroblastic growths. A return to practically normal growth energy was demonstrated during the healing stage, or period of regression of lesions. Marked sensitivity was still in evidence, however, when tuberculin was added to the culture media. Therefore the degree of sensitivity of cells from tuberculous animals to tuberculin *in vitro* does not parallel the acuity of the infectious process but represents a more or less permanent characteristic which has been impressed upon the cell as a result of the infection.

SUMMARY AND CONCLUSIONS

1. A high degree of cellular sensitivity to tuberculin toxicity was demonstrated when explants from tuberculous animals were grown in media containing that substance.

2. Similar degrees of sensitivity were noted in cells derived from animals infected with either virulent or relatively lowly virulent strains of tubercle bacilli.

3. The specificity of the tuberculin cytotoxicity was proven by testing with other bacterial cytotoxic materials.

4. Tuberculin sensitive cells grown *in vitro* in normal media showed, when tested with tuberculin, persistence of this cellular sensitivity through several transplantations during which time many new generations of cells developed.

5. There was a depression of the initial growth energy of explants

from animals during the toxic phase of the disease. During the healing stage the initial growth energy returned to normal although marked sensitivity to tuberculin persisted.

6. The degree of cellular sensitivity to tuberculin *in vitro* did not parallel the acuity of the infectious process but represented a more or less permanent acquired characteristic impressed on the cell as a result of the infection.

The authors wish to acknowledge the invaluable technical assistance of Mrs. Jessie C. Hon throughout these studies.

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EXPLANATION OF PLATES

PLATE 22

FIG. 1. Photomicrograph of splenic wandering cells or macrophages derived from a tuberculous guinea pig and growing in media containing human old tuberculin in a concentration of 1–300. 4 days after explantation. Most of the cells are dead and disintegrating. ×270. The animal had been infected 3 months previously with 5.0 mg. of strain R 1 and, at the time of the tissue culture experiment, appeared to be in excellent condition and showed no macroscopic evidence of tuberculosis except for a small caseous abscess in the inoculated testicle.

FIG. 2. Splenic macrophages from the same tuberculous guinea pig as in Fig. 1, but growing in normal media in the absence of tuberculin. 4 days after explantation. The cells are large with long filamentous pseudopodia indicating active migration. ×270.

FIG. 3. Splenic macrophages from a normal guinea pig and growing in media containing old tuberculin 1–300. 4 days after explantation. The cells are still active and healthy and the only sign of inhibition by the tuberculin is slight attenuation of protoplasmic processes. Compare with Fig. 1. ×270.

FIG. 4. Splenic macrophages from a normal guinea pig and growing in normal media showing normal active cells. ×270.

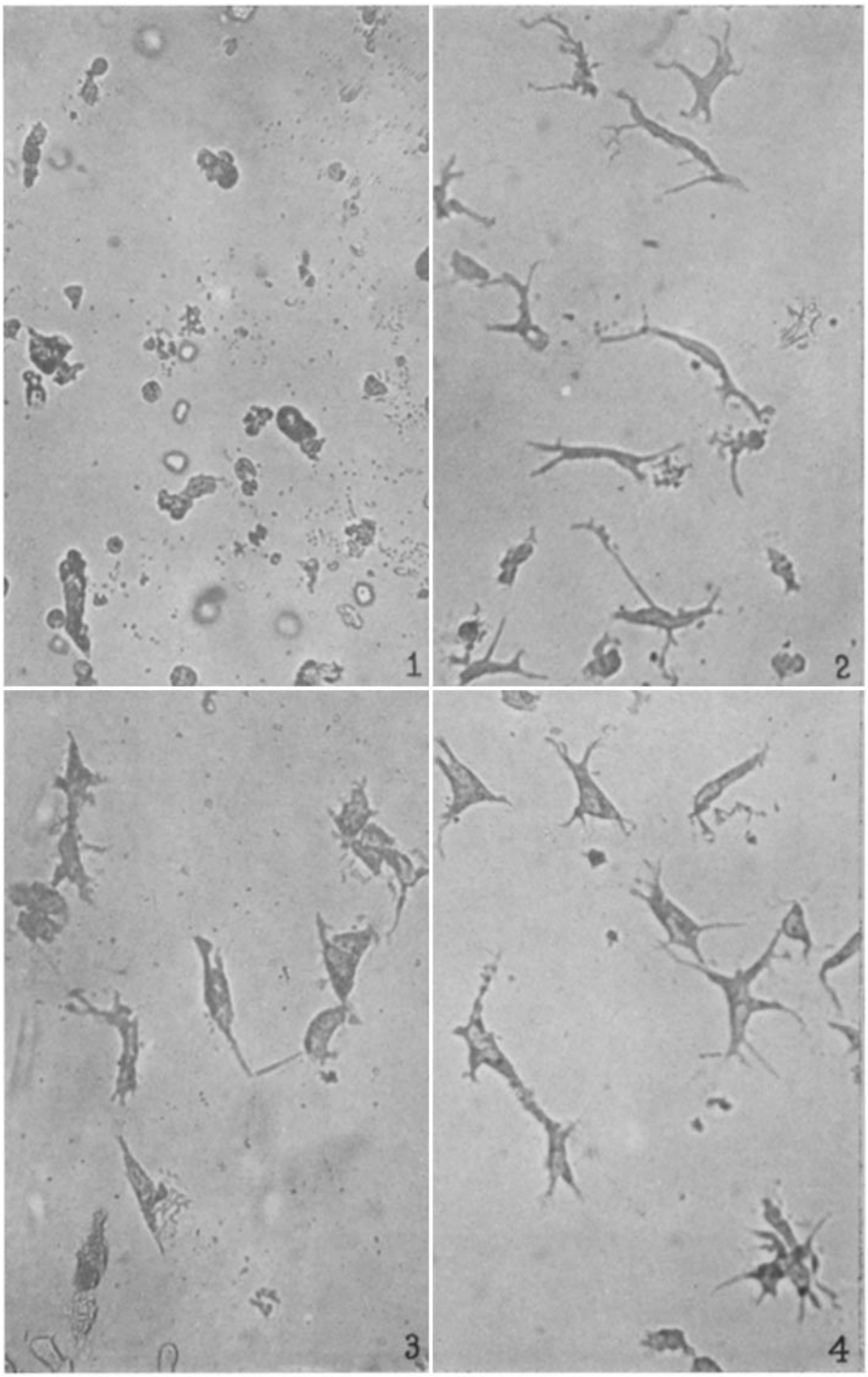
PLATE 23

FIG. 5. Lower magnification of same sensitive explant as in Fig. 1, showing marked suppression of fibroblastic growth by old tuberculin 1–300. 4 days after explantation. ×75.

FIG. 6. Lower magnification of sensitive explant in Fig. 2, showing good fibroblastic growth in normal media 4 days after explantation. ×75.

FIG. 7. Lower magnification of normal explant in Fig. 3, showing good fibroblastic growth in media containing old tuberculin 1–300. ×75.

FIG. 8. Lower magnification of normal explant in Fig. 4, showing good fibroblastic growth in normal media 4 days after explantation. ×75.



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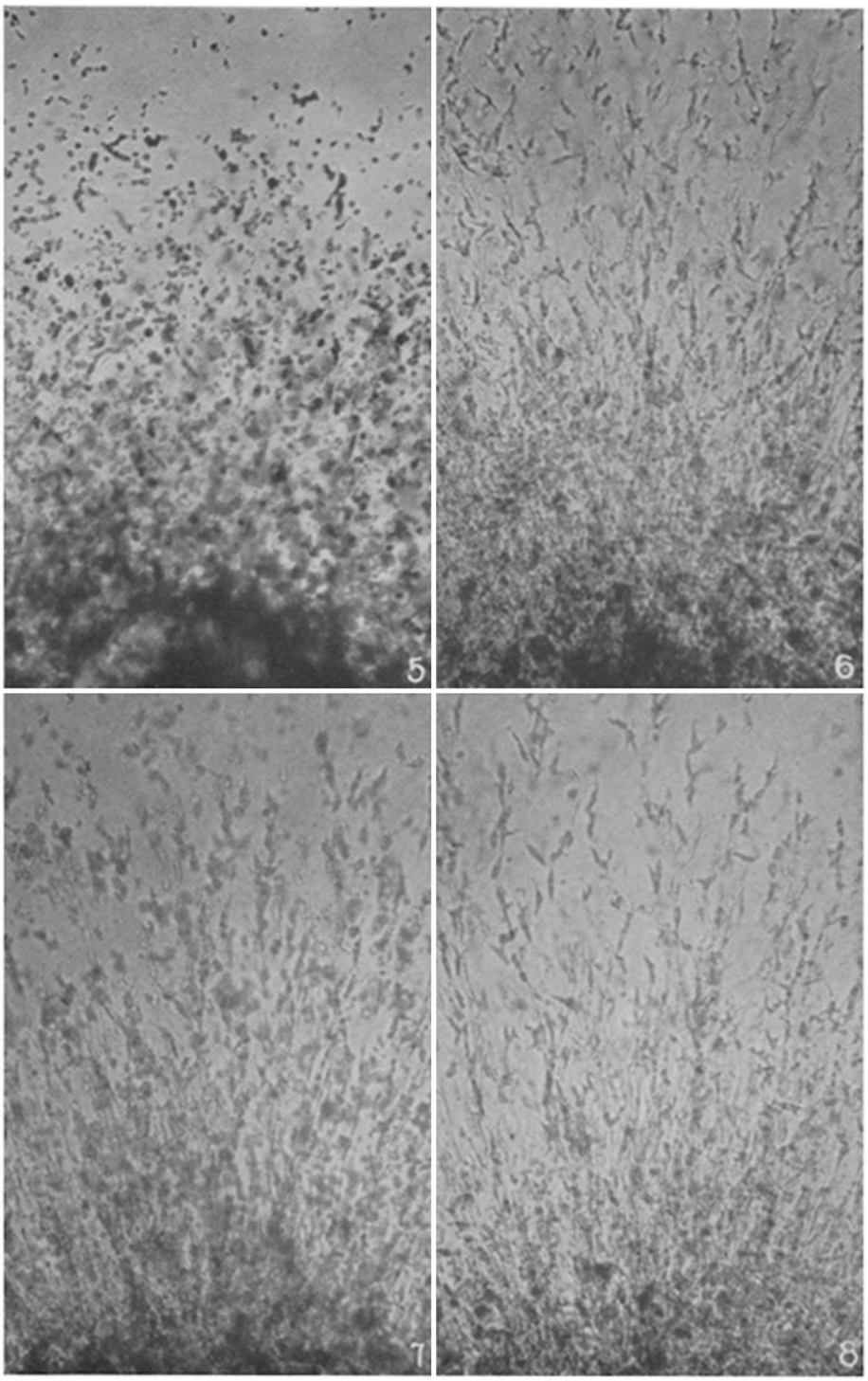

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