TABLE I.
Effect of Temperatures Above and Below Normal Incubation on Embryo and Tumor Weights of Tumor-Bearing Eggs.

| No. exp. | Incubation temperature (°F) | No. eggs inoculated | No. eggs surviving | Avg. chick size (g) | Exp. chicks, control = 100 | Avg. tumor size (g) | Exp. tumor, control = 100 |
|---|---|---|---|---|---|---|---|
| 17 (control) | 99-100 | 397 | 165 | 11.8 ± 1.8 | 100 | 1.0 ± .4 | 100 |
| 17 | 96-97 | 392 | 145 | 10.1 ± 1.2 | 85.6 | .6 ± .2 | 60 |
| 17 | 103-104 | 390 | 55 | 10.4 ± 1.0 | 88.1 | .5 ± .3 | 50 |

tumor frequently occurs in the egg together with an anemic undersized chick embryo. It appears that the relation of the chick embryo to the tumor implanted in its yolk sac is comparable to the relation of the host mouse to a tumor growing from a transplant.

It seems probable therefore that the reduction in size of the egg-grown tumors in response to temperature level above and below 99-100°F was a direct effect. Further, the data indicate that the egg-cultivated tumors are more sensitive to this factor than the supporting embryos.

## 16022

## The Tuberculin Reaction. I. Passive Transfer of Tuberculin Sensitivity with Cells of Tuberculous Guinea Pigs.*†

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(Introduced by Charles A. Evans.)

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It has been established that the "tuberculin type" of sensitivity is distinct from the anaphylactic or Arthus type of sensitivity and from the atopic type of sensitivity dependent upon Prausnitz-Küstner antibody.[1-6] Although it is probable that the tuberculin type of sensitivity depends upon an antibody, the existence of such an antibody has not been established with certainty.

That tuberculin type sensitivity is dependent upon antibody is indicated by the similarities it shows to other sensitivities known to be dependent upon antibody, namely, similarities relating to specificity, incubation time, desensitization, anamnestic reaction, and the correlation between the ability of various animal species to form ordinary antibody and to develop tuberculin sensitivity. Supporting evidence is also provided by the observation that blockage of the reticulendothelial system which depresses the formation of ordinary antibody to an injected protein, likewise suppresses the development of tuberculin sensitivity.[7] In addition, it has been found that the use of adjuvants such as

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† The term sensitivity is used throughout this article in preference to the more commonly used term hypersensitivity.

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2 Rich, A. R., Lewis, M. R., *Bull. Johns Hopkins Hosp.*, 1932, **50**, 115.

3 Rich, A. R., *The Pathogenesis of Tuberculosis*, p. 412, Charles Thomas, Springfield, Ill., 1944.

4 Aronson, J. D., *J. Immunol.*, 1933, **25**, 1.

5 Moen, J. K., and Swift, H. F., *J. Exp. Med.*, 1936, **64**, 339.

6 Heilman, D. H., Feldman, W. H., and Mann, F. C., *Am. Rev. Tub.*, 1944, **50**, 344.

petroleum jelly (Petrolatum) and paraffin oil, enhances the ability of heat-killed *Mycobacterium tuberculosis* to engender the development of tuberculin sensitivity[8,9] and, in like manner, of protein antigen to engender ordinary antibody.[10]

Many attempts have been made to accomplish passive transfer of tuberculin sensitivity.[7,11-22] It is notable that in the few reports where success has been claimed the results have been for the most part irregular and difficult to reproduce. In many instances adequate controls were not included or recognition apparently given to the difference between the true tuberculin reaction and the Arthus and anaphylactic reactions.

Helmholz[11] reported successful homologous passive transfer of tuberculin sensitivity with the defibrinated blood of tuberculous guinea pigs.

Bail[12] also reported homologous passive transfer of tuberculin sensitivity by the intraperitoneal injection of ground uncaseated spleen, liver and lymph glands of tuberculous guinea pigs. When the recipient animals were given Old Tuberculin 24 hours later by the intraperitoneal, subcutaneous, and intrapleural routes, most of the animals died within 28 to 31 hours. Bail attributed this to the systemic tuberculin reaction. Control animals given the ground spleen and liver of nontuberculous donors and injected with O.T. in the same manner survived. Cutaneous sensitivity was absent in the recipient animals. However, this is not surprising since the animals were in a very weakened condition and may have been nonspecifically non-reactive.

Joseph,[13] who attempted to repeat Bail's experiments concluded that the results observed by the latter did not prove passive transfer of tuberculin sensitivity. This was based on the observation that the tissue preparations of the donor tuberculous animals were in themselves very toxic. He assumed that the additional toxicity of the tuberculin injected may have been sufficient to kill the recipient animals without the true tuberculin reaction being involved.

Onaka[14,15] confirmed the observations of Bail and of Helmholz but did not agree with the conclusions drawn by the latter.

Massol, Breton and Bruyant[17] reported homologous passive transfer of tuberculin sensitivity with the citrated blood of tuberculous guinea pigs. They employed the technique of reversed passive transfer and used increases in body temperature as an index of tuberculin sensitivity.

Zinsser and Mueller[18] reported irregular success with heterologous passive transfer of tuberculin sensitivity to guinea pigs with the serum of tuberculous rabbits. In successful cases cutaneous reactions could be elicited 3 days following the transfer. However, positive results were infrequent and could not be reproduced at will.

Freund[7] failed to accomplish homologous local passive transfer of tuberculin sensitivity by use of blood serum and organ extracts of tuberculous guinea pigs.

Chase[22] has recently accomplished homologous passive transfer of tuberculin sensitivity in guinea pigs with living cells. The fundamental importance of this finding cannot be overemphasized. It provides the strongest

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7 Freund, J., *J. Immunol.*, 1926, **11**, 383.

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11 Helmholtz, H. F., *Ztsch. Immunitatsforschg.*, 1909, **3**, 371.

12 Bail, O., *ibid.*, 1909, Orig. IV, **1**, 470.

13 Joseph, K., *Beit. z. Klinik der Tuberk. von Brauer*, 1910, **17**, 461.

14 Onaka, M., *Z. Immunitatsforschg.*, 1910, **5**, 264.

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22 Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

evidence, thus far presented, that tuberculin sensitivity depends upon antibody and is not the result of some unknown mechanism capable of simulating antigen-antibody reactions.

Holst[1] observed that tuberculin exerts a toxic action on leucocytes derived from tuberculous animals as evidenced by decreased phagocytic power and loss of differentiation between nucleus and cytoplasm. He clearly established the difference between tuberculin sensitivity and sensitivity due to ordinary antibody by demonstrating that horse serum does not exert an *in vitro* toxic action on the leucocytes of guinea pigs sensitized to that antigen.

Rich and Lewis[2] observed that tuberculin is toxic to the cells of tissue cultures prepared from tuberculin-sensitive animals. Their work was confirmed by Aronson,[23] who noted that the toxic effect of tuberculin on tissue cultures is highly specific.

Aronson[4] made the additional observation that in contrast to this susceptibility of tuberculin-sensitive tissues, tissue cultures from Arthus-sensitive animals are unaffected by specific antigen. This was later confirmed by Rich.[24]

Knowledge of the cytotoxic effect of tuberculin on cultured cells derived from tuberculin-sensitive animals has been greatly extended by the studies of Moen and Swift[5] and Heilman, Feldman and Mann.[6]

The present report is the outcome of some of our initial studies on the nature of the tuberculin reaction. The plan of our investigations necessitated the passive transfer of tuberculin sensitivity. Hence, preliminary trials were made to accomplish passive transfer by the method reported by Chase.[22] In the earliest of these trials, it became apparent that success may be irregular, due in all probability to as yet unrecognized factors.

*Experiments.* The methods employed were essentially the same as those used by Chase,[22] except that in some of the experiments the donor guinea pigs were subjected to a superimposed infection with the B.C.G. strain of *M. tuberculosis.* The animals were secured from local sources and were of unknown pedigree. They averaged approximately 600 to 800 g in weight. In the first experiments, sensitization of the donors was accomplished by the subcutaneous injection of 25 mg (wet weight) of heat-killed *M. tuberculosis* H37 Rv in paraffin oil. In some of the subsequent experiments, the animals were sensitized by the injection of heat-killed organisms followed several months later by the intraperitoneal injection of 0.5 mg of living B.C.G. and in others by a single intraperitoneal injection of 5 to 10 mg of living B.C.G.

Five to 9 weeks following the sensitizing injections, the animals were skin-tested and the most highly sensitive animals injected intraperitoneally with 30 ml of sterile paraffin oil. Forty-eight hours later, the animals were killed and the cells of the resulting exudate were collected, washed in guinea pig serum-Tyrode solution and injected intraperitoneally into normal mature light-skinned recipient animals. Approximately 90% of the cells were alive as indicated by supravital staining with neutral red and consisted of approximately 10% polymorphonuclear cells, 47% lymphocytes, and 42% large mononuclear cells. Washed cells of the buffy coat of the minced spleens were injected intraperitoneally into other recipients. Each recipient received either the pooled peritoneal or splenic cells of from 2 to 8 donors. The cell volumes transferred to each recipient ranged from 0.4 to 1.0 ml. Control recipient animals received similar preparations from donor controls which had been given a "sensitizing" injection of paraffin oil without tubercle bacilli.

Forty-eight to 72 hours after cell transfer, all animals, including a set of normal controls, were skin-tested with O.T. in dilutions ranging from 1:10 to 1:100 or with deglycerinated O.T. employing the lowest dilution which failed to give a reaction in normal animals.

A total of 48 donors and 18 recipients were used in the various experiments. The results of the first experiment, in which donors sensitized with heat-killed tubercle bacilli were

[23] Aronson, J., *J. Exp. Med.*, 1931, **54**, 387.

[24] Rich, A. R., *The Pathogenesis of Tuberculosis*, p. 409, Charles Thomas, Springfield, Ill., 1944.

used, were negative except for a slight reaction in one recipient. The reaction in this animal became apparent after 12 hours and reached its height by 24 hours at which time it presented a 15 mm area of 2+ erythema and edema.

A careful consideration of this trial led us to believe that the most probable cause for our lack of success was that the strain of donor animals we used possessed a low ability for developing sensitivity. They showed 4+ reactions to 1:100 O.T. but only 3+ reactions to 1:100 O.T.

In an effort to render the donors more sensitive we next sensitized the animals with either heat-killed *M. tuberculosis* H37Rv and living B.C.G. or living B.C.G. alone, as outlined above. The majority of these animals developed a strong sensitivity. They reacted to 1:1000 O.T. with extensive necrosis and gave positive reactions to deglycerinated O.T. with dilutions of 1:40,000 and higher. The yield of peritoneal cells from these donors was about double that of the donors sensitized with heat-killed tubercle bacilli. Most of the recipients that received cells from these animals gave positive reactions to tuberculin. The reactions in animals sensitized with peritoneal cells were strong and of a typical tuberculin type. They were commonly negative for the first 10 hours, became fully developed by about the 30th hour and consisted of 15 mm areas of 3+ erythema, edema and induration, with a sharp central 5 mm zone of ischemic blanching. The reactions usually began to fade by the 40th to 50th hour, but were still apparent at 72 hours or longer. In several instances the early reactions were so intense that necrosis was anticipated but never developed. Intense reactions were elicited with dilution of O.T. as high as 1:100.

The animals that received splenic cells were usually less sensitive. They gave typical but less intense tuberculin reactions which lacked central blanching and faded earlier. The skin tests of most of the control animals were negative. The few controls which reacted presented small areas of 1 + erythema and edema which clearly differed from the reactions of the test animals by fading earlier and being devoid of central blanching. Fading was evident by the 24th hour and complete by the 48th hour.

The transferred cutaneous sensitivity was of short duration. Skin tests made 5 days after cell transfer were negative.

A preliminary attempt was made to elicit the systemic tuberculin reaction in two of the recipient animals by the intraperitoneal injection of 2 ml and 0.6 ml of O.T. respectively. The material was prepared for injection by dilution with several volumes of physiological saline. The animals reacted with a delayed type of shock which came on after about 3 hours. They were in severe shock by the 8th hour. The animal that received 2 ml of O.T. was dead at 24 hours and the other animal recovered. A second similar dose of O.T. administered to this animal 24 hours after the first was without effect. Normal control animals that were similarly injected remained unaffected.

*Discussion.* As Chase has emphasized, the successful passive transfer of tuberculin sensitivity in the guinea pig apparently depends upon the transfer of a large number of living leucocytes from highly sensitive donor animals.

A question of much interest and importance is whether an antibody is involved and what its nature may be. If an antibody is responsible for reactions to tuberculin, it is important to explain the apparent sessile existence of this "tuberculin antibody." Perhaps one of its properties is that it is avidly absorbed by tissues. This could account for its wide distribution and apparent sessile existence since the quantity of antibody in the circulation may thus be kept at such low levels as to be undetectable. A less-likely alternative is that all cells exhibiting sensitivity are capable of forming the antibody which remains fixed in the cells.

There appears to be little doubt that the transfer accomplished in the present experiments was passive and not active because of the short incubation period and transient nature of the sensitivity. In fact, the duration of tuberculin sensitivity for only a few days is so much shorter than the Arthus, anaphy-

lactic and Prausnitz-Kustner sensitivities as to indicate that a different and perhaps quite labile antibody must be concerned. The fact that passive transfer of tuberculin sensitivity has been accomplished only with living cells and only after an incubation period of two to three days suggests that the sensitivity may be principally due to antibody elaborated by such cells during their residence in the recipient rather than to preformed antibody.

*Summary.* Homologous passive transfer of tuberculin sensitivity was accomplished with the peritoneal and splenic cells of guinea pigs infected with the B.C.G. strain of *Mycobacterium tuberculosis.*

The possible mechanism and factors involved in passive transfer of tuberculin sensitivity are discussed.

## 16023

## Effects of X-ray Irradiation on Viscosity of Synovial Fluid.

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The treatment of arthritis by X-rays advocated in the European[1] and English[2] literature since 1930 is difficult to evaluate. However, in 1941, Smyth, Freyberg and Peck[3] demonstrated conclusively that a favorable response to X-ray therapy is obtained in ankylosing spondylitis. Many confirmatory reports have followed this observation.[4] The mechanism whereby improvement follows roentgenotherapy in such cases is not apparent.

Recently, two papers[5,6] have appeared describing a reduction in the viscosity of thymonucleic acid after irradiation, depending in degree upon the total X-ray dosage given. This is in agreement with the principles outlined by Colwell[7] in his review of work dating from 1912, to the effect that a diminution in viscosity of organic colloids such as egg-white, serum and starch followed exposure to X-ray. The reason such a change occurs is still not clear. In the case of thymonucleic acid,[6] evidence was presented that the change induced by X-radiation did not involve a splitting of primary linkages or a rearrangement of the configuration which made it susceptible to enzymatic attack.

The viscosity of normal human synovial fluid is very high.[8,9] The viscosity of joint fluids obtained from the knees of patients with rheumatoid arthritis tends to be greater in the chronic than in the acute stage, and to approach the viscosity of normal synovial fluid.[10]

The present study records observations which show that irradiation of joint fluids

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