

hours the 240 mM drop had become more dilute than the 220 mM drop. This was read as a fall below the reference solution.

It is noted that in both experiments described above (Fig. 1 and 2) the initial readings gave correct osmotic pressure values. This is in agreement with Roepke's findings⁶ on the effect of drop size on osmotic pressure determinations made with the Hill-Baldes vapor tension apparatus. He states that "it is not necessary under ordinary conditions with dilute solutions that the drops be of exactly the same size." The experiments described in the present report indicate that precautions regarding equal drop size and identical reference and wall solutions must be taken if osmotic pressure readings are to be made over long periods of time after drop deposition on the thermocouple loops. Under some conditions it may be advantageous to control these factors even when the time intervals involved are short.⁷ It is interesting

to note that the influence of these variables is exhibited in an exaggerated way when the drops consist of a mixture of deuterium oxide and water.⁸

Summary. Experiments are described that test the effect on osmotic pressure readings made with the Hill-Baldes vapor tension method, of the size of the drops placed on the thermocouple loops and of the concentration of the solution placed on the walls of the thermocouple chamber. Evidence is presented indicating that when readings are made over long periods of time after drop deposition on the thermocouple loops the drops must be of equal size and the reference and wall solutions must be identical if accurate osmotic pressure values are to be obtained throughout the course of the experiment.

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⁶ Roepke, R. R., *J. Phys. Chem.*, 1942, **46**, 359.

⁷ Lifson, N., and Lorber, V., *J. Biol. Chem.*, 1945, **158**, 209.

⁸ Lifson, N., Lorber, V., and Hill, E. L., *J. Biol. Chem.*, 1945, **158**, 219.

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The Cellular Transfer of Cutaneous Hypersensitivity to Tuberculin.

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In studies on experimental drug allergy, it has been found that specific hypersensitivity of the "delayed type" is transferable to normal guinea pigs by means of cells in exudates recovered from sensitized guinea pigs.¹ Resemblances between the delayed type of reaction to drugs and the classical tuberculin reaction have prompted investigation as to whether cells from tuberculin-sensitive animals may likewise transfer tuberculin sensitivity. The experiments show that guinea pigs receiving such cells acquire for a limited time a skin hypersensitivity that exhibits the essential features of the typical tuberculin reaction.

Guinea pigs were rendered hypersensitive

to tuberculin by subcutaneous injection of killed human tubercle bacilli suspended in paraffin oil,^{2,3} usually mixed with vaseline; each animal received 0.5 to 2.5 mg of dried tubercle bacilli in a total inoculum of 1 cc. Between 5 and 9 weeks later, the cutaneous

¹ Landsteiner, K., and Chase, M. W., *Proc. Soc. Exp. Biol. and Med.*, 1942, **49**, 688.

² Saenz, A., *C. R. Soc. Biol.*, 1935, **120**, 870, 1050; 1937, **125**, 714; *Ann. Inst. Pasteur*, 1938, **60**, 58.

³ Freund, J., Casals-Ariet, J., and Hosmer, E. P., *Proc. Soc. Exp. Biol. and Med.*, 1937, **37**, 509; Freund, J., Casals-Ariet, J., and Genghof, D. S., *J. Immunol.*, 1940, **38**, 67; Freund, J., and Gottschall, R. Y., *Arch. Path.*, 1942, **34**, 73.

reactivity to tuberculin then being pronounced, exudates were induced by the intraperitoneal injection of about 28 cc paraffin oil into each of a group of guinea pigs so sensitized. After 48 hours, the peritoneal cavities were washed out with heparinized Tyrode solution containing gelatin or normal guinea pig serum. The washings were combined and the cells recovered from the aqueous layer by minimal centrifugation. The sedimented cells were resuspended in fresh washing fluid by gentle pipetting, and again spun down. A similar washing was made, using Tyrode solution mixed with 1/10 volume of normal guinea pig serum. The washed cells were then suspended in serum-Tyrode and immediately injected into male albino guinea pigs. The yield of cells amounted to 0.1 to 0.15 cc per donor, and recipients were usually given the cells of between 2 and 10 donors. The cells were comprised of 15 to 30% of polymorphonuclear leucocytes, 20 to 35% of lymphocytes, and 50 to 65% of large mononuclear cells. The recipient animals were tested with Old Tuberculin, preferably freed of glycerine by rapid precipitation with cold alcohol, or special preparations containing tuberculo-proteins of either the larger or smaller molecular variety;* these were employed in the highest concentration that produced no reactions, or only trivial ones, in untreated control animals.

The latent period preceding the development of skin hypersensitivity varies according to the route of administration of the cells—2 to 3 days after intraperitoneal injection, and 20 to 36 hours after intravenous injection.

By way of illustration, in one experiment the cells of 9 sensitized donors were given intraperitoneally to a normal guinea pig, and the first test injections were made 2 hours later with 0.1 cc of 1:4 dilution of "deglycerinated" Old Tuberculin and with "deglycerinated" control broth in like amount; the testing was repeated on each succeeding day. Tuberculin hypersensitiveness became established in the cell recipient in about 48 hours, and exhibited maximal reactivity 72 to

96 hours after injection of the cells. The reaction occurring at the site of the second test injection of tuberculin developed thus:

24 hours: 8 mm (in diameter) faint pink; 3 mm white center; outer diffuse color.

48 hours: 13 mm pale pink, diffuse margin; moderately thickened; 8 mm livid area with 4 mm white center.

72 hours: 16 x 12 mm diffuse faint pink; indurated; 9 mm white center with spotty pink margin; 4 mm brownish necrotic central zone.

The reaction 48 hours after the third test injection with tuberculin was somewhat weaker than that developed during the same interval by the second test injection. After the 5th day, the control animals exhibited an active sensitization to the tuberculin preparation—largely a sensitivity to the broth medium—and the experiment was terminated. This effect is readily differentiable from the typical tuberculin reaction, and can be attained regularly by injecting control broth into normal guinea pigs for 4 to 6 days. Necrosis and sloughing of the test sites occurred in the cell recipient only.

In contrast to the reactions following upon the injection of washed cells, similar effects have not been obtainable with serum of the donors of active cells, or with cells from normal animals.

Of 17 experiments of the sort described, successful transfers have been obtained in 16 instances. The intensity of the transferred hypersensitiveness has varied in accordance with several factors—chiefly the amount of cells used, and the degree of sensitivity of the cell donors.

It has been established that the cells become largely or entirely inactive upon being heated at 48°C for 15 minutes or upon freezing, and are markedly less active after being kept in the icebox over night. The duration of the transferred sensitivity appears to be brief. In addition to exudate cells from the peritoneal cavity, cells from the spleen or lymph nodes are capable of transferring hypersensitiveness to Old Tuberculin. The brief duration of sensitivity and the activity of cells from peritoneal exudates, spleen, and lymph nodes parallel our experiences in the transfer of drug allergy.

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