TABLE I.
Activity of C^{14} in Tissue Proteins in Counts per mg per Minute.*

	Tryptophan deficiency		Phenylalanine deficiency			
	C^{14}-Tryptophan administered		C^{14}-Tryptophan administered		C^{14}-Glycine administered	
Organ	Supplemented	Deficient	Supplemented	Deficient	Supplemented	Deficient
Intestines	64.8	28.9	43.9	38.8	197	174
Plasma	52.0	28.0				
Liver	41.5	27.9	20.3	17.7	140	105
Kidney	34.9	21.4	29.7	20.6	118	68.0
Spleen	22.7	9.6	28.2	17.5	114	90.2
Testes	19.6	5.4				
Stomach	30.3	8.0				
Heart	10.5	4.3	8.4	5.1	43.0	29.4
Brain	10.2	4.3				
Skeletal muscle	4.7	2.7	4.5	1.8	23.4	5.0
Red cells	5.0	2.0				

* The data represents the average value of 2 animals in each group.

ficiency both when the animals are starved or fed. The decrease may be due to a decrease in net synthesis of protein or to a lowered rate of turnover, or to both combined. The deficiency of the amino acid could directly limit the quantity of enzymes responsible for the incorporation of the label or the effect may be an adaptive response.

Summary. Animals reared on a diet deficient in an essential amino acid (tryptophan, phenylalanine) showed a reduced incorporation of tracer amounts of C^{14} from labeled amino acids in the tissue proteins.

16653

Passive Transfer of Local Cutaneous Hypersensitivity to Tuberculin.

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Many attempts have been made in the past to transfer tuberculin hypersensitivity passively to normal animals. Most of these attempts have been unsuccessful, and the positive results claimed by some authors have either been seriously contested by others, or have been too irregular to be quite convincing.[1,2] A method which insures a readily reproducible transfer of tuberculin hypersensitivity was first described by Chase[3]; this method had been previously devised by Landsteiner and Chase[4] for the passive transfer of the "delayed type" of skin sensitivity to simple chemical compounds. Briefly, it consists in injecting a suspension of living exudate, spleen or lymph node cells from actively sensitized guinea pigs into the peritoneal cavity or the blood stream of normal animals, following which the latter develop general cutaneous hypersensitiveness to tuberculin within 1 to 3 days. Using the same or slightly modified methods, Kirchheimer and Weiser,[5] Cummings, Hoyt, and Gottshall,[6]

1 Zinsser, H., and Mueller, J. H., *J. Exp. Med.*, 1925, **41**, 159.

2 Rich, A. R., The Pathogenesis of Tuberculosis, p. 398, 2nd printing, Springfield, Charles C. Thomas, 1946.

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

4 Landsteiner, K., and Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 688.

5 Kirchheimer, W. F., and Weiser, R. S., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 166.

and Stavitsky[7] have obtained similar results. The object of the present study was to determine whether passive tuberculin hypersensitivity can also be induced locally by the intracutaneous injection of exudate cells from tuberculin sensitive animals.*

The methods were based on those used by Chase,[3] and adapted to the special purpose of this work outlined above. Guinea pigs weighing from 550 to 950 g were used as cell donors. They were infected with a recently isolated virulent human strain of *M. tuberculosis*, by the subcutaneous injection of 0.2 mg (moist weight) of bacilli in saline. 3½ to 7 weeks later the animals were skin-tested with O.T. before each experiment, and those showing the strongest reactions were selected for the production of peritoneal exudate. Each experiment comprised 2 tuberculous and 2 normal cell donors. All 4 animals received 30 cc of paraffin oil intraperitoneally. Forty-eight hours later they were killed, and the peritoneal cavities were washed out with heparinized Tyrode solution. The "tuberculous" and the "normal" exudate cells were recovered from the washings separately by mild centrifugation and washed three times in fresh Tyrode. Finally they were made up with heparinized Tyrode into very thick suspensions, similar in appearance and consistency to slightly diluted pus. 0.1 cc of "tuberculous" and of "normal cell" suspension was then injected intracutaneously into each flank of a young albino guinea pig, 3, 4 or 5 such cell recipients (between 250 and 400 g in weight) being used in each experiment. A few drops of the cell suspension left in the injection syringe were filled into a short capillary tube, which was then centrifuged at 2,500 r.p.m. for ½ hour. In this tube the cell volume/suspension volume ratio could be accurately determined; it amounted to about 2/5, and as it remained constant on further sharp centrifugation, it was adopted as a reliable basis on which to calculate the cell volume actually injected. Thus each cutaneous bleb contained 0.035-0.045 cc of cells, the difference in volume between "tuberculous" and "normal" cells in any particular experiment never being more than 0.005 cc. The cells consisted of 57-75% large mononuclear cells, 8-36% lymphocytes, and 6-22% polymorphonuclear leucocytes, the "tuberculous" cells usually comprising more lymphocytes and less polymorphonuclears than the "normal" cells.

The injection of cell suspension elicited a marked reaction in the skin of the recipients; this consisted, 48 hours after the injection, in a moderately indurated circular area of erythema measuring between 5 and 9 mm in diameter, with a small yellowish center, rarely wider than 1 mm. At this time the recipients were tested with tuberculin. For this purpose two technics were employed: a) the direct injection into the prepared skin sites of 0.1 cc O.T. 1/10; and b) the injection at a distant point (intraperitoneally or subcutaneously) of 1.0-1.5 cc O.T., diluted in an equal volume of saline. The first method was not quite satisfactory, as the control sites (prepared with "normal" cells) reacted to the direct introduction of tuberculin with a slight increase in swelling and redness. This was entirely avoided with the second method ("Fernauslösung"), which was adopted for most of the experiments. The intraperitoneal injection of O.T., however, in the amounts mentioned above, resulted in every case in a fairly severe (but never lethal) anaphylactoid shock. On the other hand, the subcutaneous injection of 1.5 cc of undiluted O.T. was unsatisfactory, owing to protracted absorption of the depot, which remained visible through the abdominal skin for several days. It was felt that the severity of shock was somewhat reduced by the following combination of both routes of administration: 0.25 cc O.T. + 0.25

[6] Cummings, M. M., Hoyt, M., and Gottshall, R. Y., *Pub. Health Rep.*, 1947, **62**, 994.

[7] Stavitsky, A. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 225.

* H. J. Corper and R. E. Stoner have also attempted to induce passive local hypersensitiveness to tuberculin in guinea pigs by the intracutaneous injection of a mixture of tuberculin and citrated whole blood from actively sensitized animals (*Amer. Rev. Tub.*, 1946, **54**, 305). Their uniformly negative results were probably due to the low proportion of white cells in whole blood, the relatively high dilution of tuberculin used, and the simultaneous injection of both reagents.

cc saline subcutaneously, and 15 minutes later 1.25 cc O.T. + 1.25 cc saline intraperitoneally. Most of the "Fernauslösung" experiments were carried out in this manner.

The results of both types of experiment were clear-cut. The reactions at the skin sites prepared with "tuberculous" cells were well developed 16 hours after tuberculin testing and usually at their height at 24 hours. At this time they presented the aspect of rather small but characteristic tuberculin reactions: an indurated, blanched or livid area (4-9 mm in diameter), surrounded by a diffuse zone of erythema significantly wider than before the injection of tuberculin (10-15 mm). In several instances the center was hemorrhagic, thus completing the picture to that of a typical "cocarde" reaction. Strongly reacting sites became necrotic after 48 to 72 hours, and sloughed off in the next few days. It should be noted that all the reactions were of the delayed type; occasionally very slight blanching of a sensitized site was seen as early as 7 hours after the O.T. injection, but never any earlier. In contrast, no change whatever was observed in the control sites after the introduction of tuberculin at a remote point, and, as has already been mentioned, only a slight increase in inflammatory symptoms was seen after local tuberculin testing.

By way of illustration in one experiment 4 young albino guinea pigs received 0.039 cc "tuberculous" cells intracutaneously in the right flank, and 0.042 cc "normal" cells in the left. The sequence of reactions in one of these animals was as follows:

Forty-eight hours after the cell injections: in both flanks areas of swelling and redness (6x6 mm) with a small yellowish center (1x1 mm). 0.25 cc O.T. + 0.25 cc saline are injected under the abdominal skin, followed 15 minutes later by 1.25 cc O.T. + 1.25 cc saline i.p. Immediate shock of moderate intensity; recovery complete in a few minutes. Twenty-four hours later the inflamed area in the left flank is unchanged. Reaction in the right flank: 8x9 mm white indurated area, sharply demarcated, with 4x5 mm violet-red (hemorrhagic) center; outer zone 10x12 mm angry red diffuse margin. Forty-eight hours after tuberculin: 6x8 mm black necrotic center, 10x12 mm red outer zone; the former sloughed off in a few days.

Five experiments of the kind described above were performed, involving a total of 41 animals, 21 of which were used as cell recipients. In 15 of these, transfer of tuberculin hypersensitivity was successful, as evidenced by medium to strong tuberculin reactions at the specifically prepared skin sites. In one experiment an attempt was made to determine whether local sensitivity is preceded by a latent period: the 5 recipients used, including one animal tested 48 hours after the cell injections, gave negative or at best only suggestive reactions, and had to be recorded as failures. In another experiment the duration of local reactivity was explored; this was found to last up to 5 days, and the one recipient in the experiment which was tested 7 days after sensitization failed to react at all. Tests carried out with control glycerine broth gave entirely negative results, whether the broth was injected locally into prepared skin sites or intraperitoneally (when it also caused anaphylactoid shock).

Conclusions. The method of local passive sensitization so extensively employed in human allergy (Prausnitz-Küstner reaction) and in experimental anaphylaxis (Opie and others), can also be applied to the cellular transfer of tuberculin hypersensitivity discovered by Chase.[3] Compared with the latter's experimental procedure, this method offers the advantage of considerably reducing the number of cell donors necessary for successful transfer. The technic used is, however, susceptible of improvement, as in the presence of a marked primary reaction of cavy skin to the injection of homologous exudate cells none but fairly strong tuberculin reactions can be recorded with confidence.