ly-spaced parallel bands or densities. The organization of these is usually in the form of a fiber (Fig. 4 and 5), but occasional patches of irregular outline have been observed. The periodicity displayed measures as a rule between 1000 to 1100 Å. The densities in shadowed preparations appear as prominent elevations and there may be extremely little if any material connecting them. There is no evidence of intra-period banding. In most of the collagen preparations examined, this component has been found scattered rather sparsely among the fibers, but it has also been observed without associated fibers. We are therefore unable to decide whether it is related in any way to collagen formation, but are inclined to the view that it represents a component of the connective tissue ground substance.

The available evidence indicates that the major fibrous components of these preparations, exclusive of the last, are collagen. The periodicity of the large compound fibers is like that of collagen, the fibers resist tryptic digestion, and staining technics have identified collagen fibers in cultures paralleling these used for electron microscopy.

The sequence of events leading to mature fiber formation, while not directly observable, can be reasonably reconstructed from the character and associations of the various components of any fibrous array. Thus the relation of the unit fibers to the larger strands is clear; they are obviously the component units and appear to have come together into these parallel arrays much the same as unit fibers of fibrin aggregate to produce the larger strands of the formed clot.[9] But the origin of the unit fibers is less evident. They are apparently not spun off the cells. Instead, it is probable that they are formed, as in fibrin, through a lateral association of several protofibrils and a progressive deposition of molecular collagen on their surfaces.[10] Since the more slender unit fibers show a fine even periodicity, it appears that the larger collagen pattern of the mature fiber is a secondary development. These and other phenomena of connective tissue fiber formation will be considered more completely in a later report.

[9] Hawn, C. V. Z., and Porter, K. R., *J. Exp. Med.*, 1947, **86**, 285.

[10] Porter, K. R., and Hawn, C. V. Z., *J. Exp. Med.*, 1949, **90**, 225.

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17242. The Cellular Transfer of Cutaneous Hypersensitivity to Tuberculin in Man.

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It has been demonstrated by Chase[1] that transfer of specific cutaneous hypersensitivity of the "delayed type" to tuberculin can be accomplished in unsensitized guinea pigs by the injection of leucocytes isolated from peritoneal exudates produced in sensitized guinea pigs.

It is the purpose of this report to describe the transfer of cutaneous hypersensitivity to tuberculin in man by the intradermal injection of viable leucocytes isolated from the peripheral blood of non-tuberculous humans.

Materials and Methods. Utilizing the method of Minor and Burnett[2] it has been possible to isolate and concentrate viable leucocytes from human blood by the addition of bovine fibrinogen, Fraction I (Armour), which accelerates the rate of erythrocyte sedimentation leaving a plasma suspension of leucocytes in the supernatant portion.

[1] Chase, M. W., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 134.

[2] Minor, A. H., and Burnett, L., *J. Hematology*, 1948, **7**, 799.

1. *Technic of obtaining leucocytes.* Whole venous blood was drawn and placed in sterile potato tubes containing 0.4 ml of a 100 mg % heparin solution for each 10 ml of blood. The tubes were inverted twice and 1.0 ml of sterile Seitz filtered bovine fibrinogen, Fraction I (Armour) solution, (containing approximately 45 mg fibrinogen per ml) was added for each 10 ml of blood and the tubes inverted twice. The heparinized and fibrinogenized blood was then transferred in 10 ml aliquots to conical centrifuge tubes which were placed in a water bath at 37°C for 1 hour. At this time the erythrocytes have become packed in the lower half of the tube and a suspension of leucocytes is contained in the supernatant plasma. The latter was transferred by capillary pipette to specially constructed 10 ml capillary-tip centrifuge tubes (Machlett, No. A 17-210) and centrifuged in an angle centrifuge at 3000 RPM for 1 hour. The cell-free plasma was then decanted and the packed leucocytes (average volume packed wet cells from each 10 ml plasma suspension = 0.025 ml) were resuspended and washed in either 2.0 ml of Tyrodes solution (pH 7.8-8.3) or 2.0 ml of freshly drawn serum obtained from the tuberculin negative recipient of the cells. The cell suspensions were collected from each tube and pooled in one capillary-tip centrifuge tube to a volume of 10 ml and centrifuged again at 3000 RPM for 30 minutes. The supernatant portion was decanted and the washing and centrifugation of the leucocytes was repeated. The supernatant portion of the second washing was decanted, the volume of packed leucocytes read, and then resuspended in either 1.0 ml of Tyrodes solution or 2.0 ml of serum. This suspension of twice washed leucocytes was then injected intradermally into the tuberculin negative recipient.

The interval between the withdrawal of blood and the injection of leucocytes into the recipient was usually not greater than 6 hours. The leucocytes were not refrigerated or stored and rigid sterile technics were observed in the procedure described.

2. *Dosage of leucocytes and vehicle for suspension.* Either 60 ml or 100 ml of venous blood was obtanied from each tuberculin positive donor with normal total peripheral and differential leucocyte count.

The average volume of packed wet leucocytes isolated from 60 ml whole blood was 0.07 ml. The leucocytes were twice washed with 10 ml of Tyrodes solution (pH 7.8-8.3) and resuspended in 1.0 ml of Tyrodes solution for intradermal injection.

The average volume of packed wet leucocytes isolated from 100 ml whole blood was 0.20 ml. The leucocytes were twice washed with 10 ml of serum and resuspended for intradermal injection in 2.0 ml of serum freshly drawn from the tuberculin negative recipient.

3. *Tuberculin Materials Used in Test.* The subjects were tested with Old Tuberculin (O.T.) or Purified Protein Derivative (PPD). The leucocyte donors reacted to either 0.1 ml (1.0 mg) O.T. or to 0.1 ml (0.005 mg) PPD intradermally with +++ to ++++ reactions after 48 hours. The recipients of the leucocytes had no reaction to either O.T. (1.0 mg) or PPD (0.005 mg) intradermally after 48 hours.

4. *Selection of Donors and Recipients.* The group is comprised of 11 tuberculin negative recipients and 5 tuberculin positive donors. The donors and the recipients of the leucocytes were patients hospitalized on the wards of the Third (NYU) Medical Division of Bellevue Hospital or were normal young adults and ranged in age from 21 to 65 years. They were not suffering from any intercurrent infection, were neither cachectic nor myxedematous and had no clinical, laboratory or roentgenographic evidence of active pulmonary or systemic tuberculosis.

5. *Method of Leucocyte Transfer.* Two methods of leucocyte transfer were used.

a) *Prausnitz-Küstner Passive Transfer of Leucocytes.* Using the passive-transfer method of Prausnitz-Küstner, 1.0 ml of a Tyrode suspension of viable leucocytes obtained from a tuberculin positive donor was injected intradermally into the flexor surface of the forearm of the tuberculin negative recipient. After an interval of 18, 24 or 48 hours the reaction at the site of cell transfer was

TABLE I.
Cellular Transfer of Cutaneous Tuberculin Hypersensitivity to Tuberculin Negative Recipients, Using the Method of Prausnitz-Küstner.

Tuberculin negative recipient	Tuberculin reaction*† at WBC site—48 hr	Tuberculin reaction distant from WBC site—48 hrs	Duration of induced tuberculin positive state	Tuberculin status of recipient at present
S.D.	++++	+++	>2 mo.	Unknown
E.K.	++++	+++	1 "	Negative
A.G.‡	+++	+++	1 "	"
A.G.‡	Not challenged at WBC site	++	1 wk	"
S.P.	++	+++	2 "	"
N.P.	++	++++	>1 mo.	Unknown

* *Criteria for reading the intradermal tuberculin reaction:* The criteria for reading the intradermal tuberculin reaction were the same for all individuals studied:

(a) Tuberculin Negative Reaction: Recorded when no reaction occurred at the intradermal site of P.P.D. 0.1 ml (0.005 mg) or O.T. 0.1 ml (1.0 mg) after 48 hours.

(b) Tuberculin Positive Reaction: Graded according to the severity of the reaction at the intradermal site of P.P.D. 0.1 ml (0.005 mg) or O.T. 0.1 ml (1.0 mg) after 48 hours, as outlined below:

\+ —reactions more than 5 mm and not exceeding 10 mm in diameter, showing some redness and definite edema.

++ —reactions more than 10 mm but not exceeding 20 mm in diameter, with an area of redness and edema.

+++ —reactions more than 20 mm but not exceeding 30 mm with marked redness and edema.

++++—reactions exceeding 30 mm in diameter with marked redness and edema.

† The tuberculin preparation used throughout this series of observations was Old Tuberculin (O.T.).

‡ Same tuberculin negative recipient.

measured and the cell site and a control site some distance from the latter were challenged with 0.1 ml (1.0 mg) O.T. intradermally. Reactions were read in both sites at 15 and 30 minutes and 24, 48, 72 and 96 hours. Three subjects were given additional control intradermal injections of an equal volume of erythrocytes and of serum from the same donor and the sites challenged as above.

b) *Method of Tuberculin Challenge at a site distant from the cell site.* A 2.0 ml serum suspension of leucocytes was injected intradermally in the deltoid area of the tuberculin negative recipient and after an interval of 18 hours, only the skin of the forearm was challenged with 0.1 ml (0.005 mg) PPD intradermally. Readings of the tuberculin reaction in the forearm were made as outlined above.

6. *Control Observations.* Three subjects were given leucocytes from tuberculin negative donors and challenged with tuberculin in the manner described immediately above. These subjects remained tuberculin negative, subsequently were then given leucocytes from tuberculin positive donors.

The first recipient, S.D., became tuberculin positive at the site of leucocyte transfer but not in the control site prepared with donors serum nor in a site distant from both above sites. After a 16 day interval S.D. was given a second injection of leucocytes from the same tuberculin positive donor and 24 hours later the site of earlier leucocyte transfer, which had faded entirely, the new site of leucocyte transfer and a site distant from both, were each challenged with tuberculin. As is shown in Fig. 1, this recipient developed positive reactions to tuberculin in the old cell site, the new cell site and for the first time in a site distant from the cell sites. This was the first indication that a systemic as well as a local alteration of cutaneous reactivity to tuberculin occurred following leucocytic transfer.

All of the leucocyte recipients who received control intradermal injections of serum and of erythrocytes from tuberculin positive donors, developed positive reactions at the site of leucocyte transfer but not at the sites of serum or of erythrocyte transfer when each site was challenged with O.T. (1.0 mg). The negativity at the sites of serum and erythrocyte transfer persisted until general cutaneous hypersensitivity to tuberculin appeared.

Three recipients (B.H., M.P., R.G.) were injected intradermally with leucocytes obtained from tuberculin negative donors in

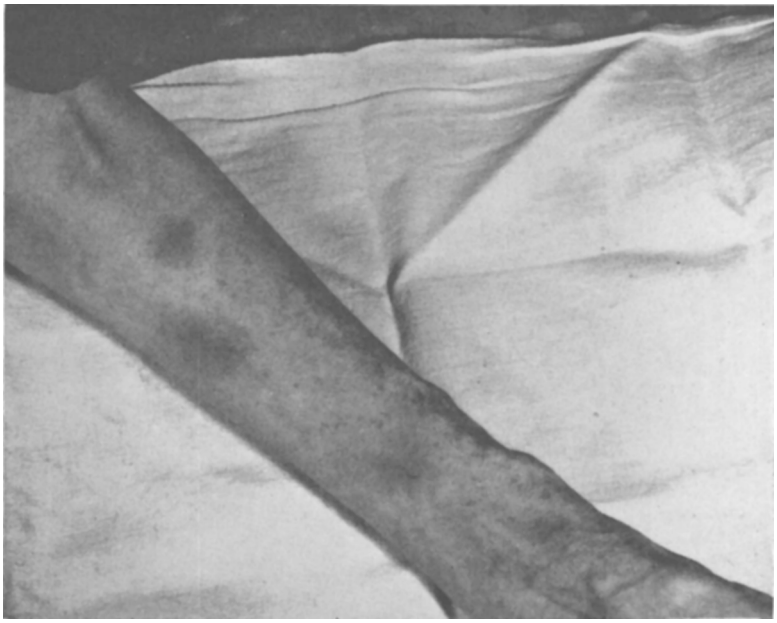

FIG. 1.
Induced Tuberculin Positive Reaction.
Subject S.D. at 24 hr.
Upper: Old cell site challenged with O.T. 1.0 mg.
Middle: New cell site challenged with O.T. 1.0 mg.
Lower: Distant site challenged with O.T. 1.0 mg.

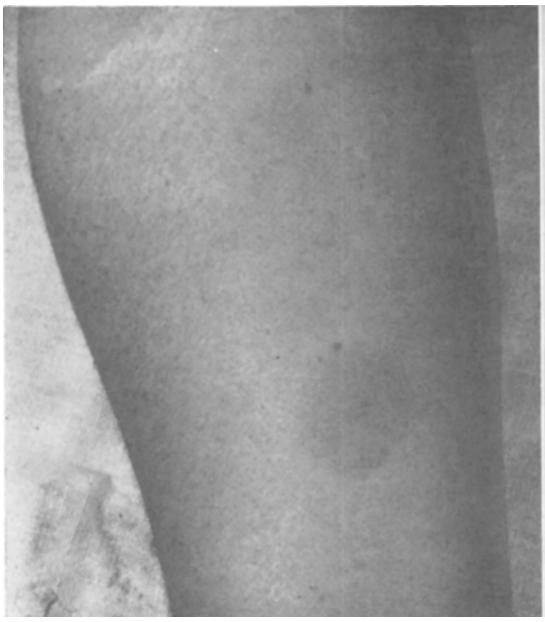

FIG. 2a.
Induced Tuberculin Positive Reaction.
Subject E.K. at 24 hr.
Site distant from cell site challenged with O.T. 1.0 mg.

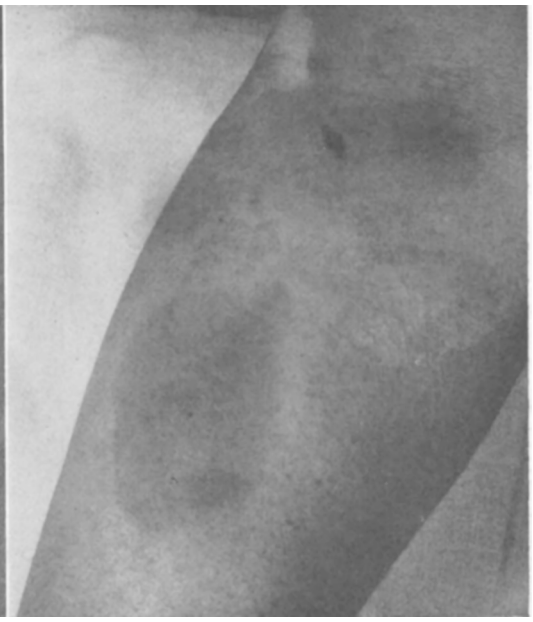

FIG. 2b.
Induced Tuberculin Positive Reaction.
Subject E.K. at 24 hr.
Cell site challenged with O.T. 1.0 mg.

TABLE II.
Cellular Transfer of Cutaneous Tuberculin Hypersensitivity to Tuberculin Negative Recipients, Using the Method of Tuberculin Challenge at a Site Distant from the Cell Site.

Tuberculin negative recipient	Tuberculin* status of leucocyte donor	Volume of packed WBC, ml	Tuberculin reaction after WBC transfer at 48 hours	Duration of induced tuberculin positive state	Tuberculin status of recipient at present
B.H.	Negative	0.04	0		
	Positive	0.05	0		
	"	0.10	+	4 days	Negative
M.P.	Negative	0.04	0		
	Positive	0.05	0		
	"	0.10	++	3 mo.	"
R.G.	Negative	0.20	0		
	Positive	0.20	++++	Unknown	Positive
P.O.	"	0.15	+++	>1 mo.	"
S.S.	"	0.20	+++	>1 "	"
A.S.	"	0.20	++	>1 "	"

* The tuberculin preparation used throughout this series of observations was Purified Protein Derivative (PPD).

order to determine whether the presence of the cutaneous tuberculin hypersensitivity of the leucocyte donor is necessary for the successful transfer of that hypersensitivity to the recipient. In each instance the passive transfer of leucocytes obtained from a tuberculin negative donor had no effect upon the tuberculin reaction of the tuberculin negative recipient. However, the subsequent passive transfer of leucocytes obtained from tuberculin positive donors given to the same three tuberculin negative recipients was followed by the development of cutaneous tuberculin hypersensitivity in each.

It appeared possible but unlikely, that the manipulation or the materials used in the isolation and concentration of the leucocytes, may have directly caused or indirectly contributed to the transfer of cutaneous hypersensitivity to tuberculin. To explore this possibility, leucocytes were obtained from a tuberculin negative donor (M.P.) and the leucocyte suspension injected intradermally into the same tuberculin negative donor (M.P.). This procedure had no effect upon the cutaneous reaction to tuberculin in this individual, whereas the subsequent transfer of leucocytes obtained from a tuberculin positive donor was followed by the development of tuberculin hypersensitivity.

The observations made on subjects (B.H.) and (M.P.) suggest the probable importance of the actual amount or dosage of the packed leucocytes transferred, in determining the development of tuberculin hypersensitivity in the individual recipient. The volume of packed leucocytes obtained from a ++++ tuberculin positive donor was 0.1 ml, half of which (0.05 ml) was given to tuberculin negative recipient (B.H.) and half (0.05 ml) given to tuberculin negative recipient (M.P.). Both recipients remained tuberculin negative when subsequently challenged with tuberculin. The transfer of leucocytes obtained from another ++++ tuberculin positive donor was repeated, this time 0.1 ml of packed cells was injected into each recipient (B.H. and M.P.). Subsequent challenge with tuberculin resulted in the development of a positive reaction in each recipient.

Of interest is the phenomenon observed in subject P.O. (Fig. 3). This tuberculin negative recipient had no reaction to PPD (0.005 mg) for 72 hours and then 6 hours after the transfer of leucocytes from a tuberculin positive donor, developed a positive reaction at the formerly negative PPD site, which reached its maximum intensity (++) at 48 hours. When challenged with PPD (0.005 mg) 24 hours after leucocyte transfer, this individ-

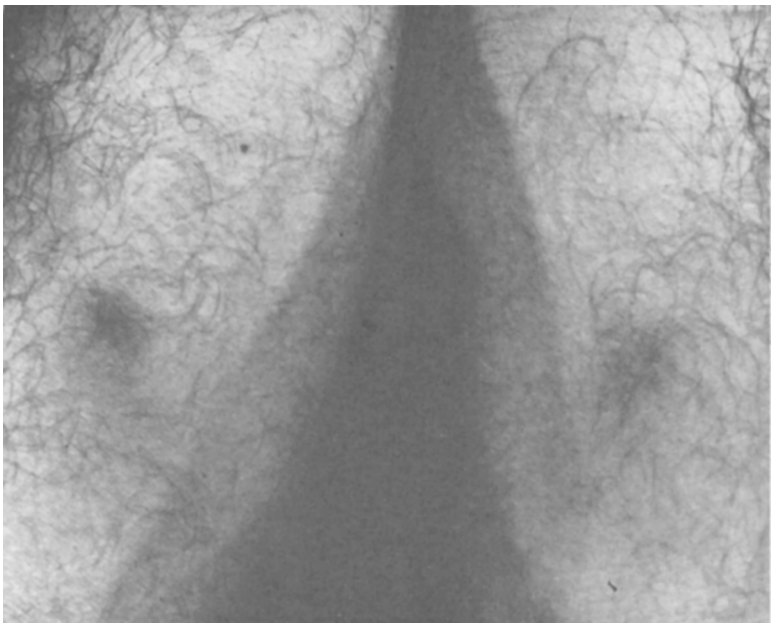

FIG. 3.
Induced Tuberculin Positive Reaction Subject P.O.
Left: Reaction at 48 hours (PPD-0.005 mg) at site distant from cell site, which had been challenged 24 hours after leucocyte transfer.
Right: Reaction at 72 hours (PPD-0.005 mg) at site distant from cell site, which had been negative until 6 hours after leucocyte transfer.

ual developed a positive reaction at the new PPD site which reached its maximum intensity (+++) at 48 hours. The delayed appearance of a positive reaction at the site of a formerly negative tuberculin test has been commented upon by several observers[3] and it usually occurs secondary to the development of a primary tuberculous infection in the interim between testing and the appearance of the positive reaction. There has been no clinical or roentgenographic evidence that a primary tuberculous infection had occurred in subject (P.O.). To substantiate this, is the observation that the degree of his induced cutaneous hypersensitivity to tuberculin has decreased progressively in intensity from a (+++) to a (+) reaction in the month following leucocyte transfer.

The observations tabulated in Table III suggest the probable importance of the degree of tuberculin hypersensitivity of the leucocyte donor, in determining the degree of induced tuberculin hypersensitivity of the individual recipient.

Of the 6 tuberculin negative recipients who were followed with repeated tuberculin testing after conversion to the tuberculin positive state, 5 spontaneously reverted to tuberculin negativity after a variable period of 4 days to 3 months.

One of the subjects (A.G.) with induced cutaneous hypersensitivity to tuberculin who spontaneously reverted to a tuberculin negative state, was sensitized again with leucocytes obtained from another tuberculin positive donor and subsequently became tuberculin positive for the second time. This subject has since become tuberculin negative again.

The recipients P.O., S.S., A.S. and R.G., with induced cutaneous tuberculin hypersensitivity of more recent origin, are still tuberculin positive at present. However, the degree of tuberculin hypersensitivity of P.O. and S.S. has decreased progressively in intensity from a (+++) to a (+) reaction in the month following leucocyte transfer. The observations

[3] Daniels, M., *Lancet*, 1943, **245**, 600.

TABLE III.
Degree of Induced Tuberculin Hypersensitivity in Relation to Degree of Hypersensitivity of Leucocyte Donor.

Tuberculin positive donor	Degree of tuberculin hypersensitivity of donor	Tuberculin negative recipient per donor	Degree of tuberculin hypersensitivity in recipient following WBC transfer	Duration of tuberculin positive state	Tuberculin status at present
		S.D.	+++	>2 mo.	Unknown
		E.K.	+++	1 "	Negative
D.M.	+++	B.H.	+	4 days	"
		M.P.	++	3 mo.	"
		A.G.†	+++	1 "	"
C.S.	++++	S.P.	+++	2 wk	"
		N.P.	++++	>1 mo.	Unknown
P.B.	++++	A.G.†	++	1 wk	Negative
		P.O.	+++	1 mo.	Positive*
R.H.	++++	S.S.	+++	1 "	" *
		A.S.	++	1 "	" *
		R.G.	++++	Unknown	" *

* Each recipient still under observation.
† Same tuberculin negative recipient.

made on the recipients with induced cutaneous tuberculin hypersensitivity, who spontaneously reverted to a tuberculin negative state, indicated that the progressive decrease in the intensity of the tuberculin reaction was followed shortly thereafter by the return to tuberculin negativity.

Discussion. In twelve consecutive instances the tuberculin negative recipients of viable leucocytes obtained from tuberculin positive donors, subsequently developed cutaneous hypersensitivity to tuberculin. When the leucocytes were obtained from tuberculin negative donors, cutaneous hypersensitivity to tuberculin did not subsequently develop in the tuberculin negative recipient.

The cutaneous hypersensitivity to tuberculin following leucocyte transfer is a transient phenomenon of 4 days to 3 months duration. The induced cutaneous hypersensitivity to tuberculin can be produced again in the same individual.

The degree of induced hypersensitivity to tuberculin seems to bear a relationship to the dosage of leucocytes used and the degree of tuberculin hypersensitivity of the leucocyte donor.

These observations in man are in agreement with those made by Chase[1] in the guinea pig.

Summary and conclusions. 1. It has been possible to passively transfer in 12 consecutive instances cutaneous tuberculin hypersensitivity to tuberculin negative human recipients by means of an intradermal injection of viable leucocytes obtained from the blood of tuberculin positive human donors.

2. The effort at the passive transfer of cutaneous tuberculin hypersensitivity was unsuccessful when the leucocytes used in the transfer were obtained from tuberculin negative donors.

3. The induced cutaneous hypersensitivity to tuberculin is a transient phenomenon of 4 days to 3 months duration, which can be produced again in the same individual.

4. The degree of induced hypersensitivity to tuberculin seems to bear a relationship to the dosage of leucocytes used and the degree of tuberculin hypersensitivity of the leucocyte donor.

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