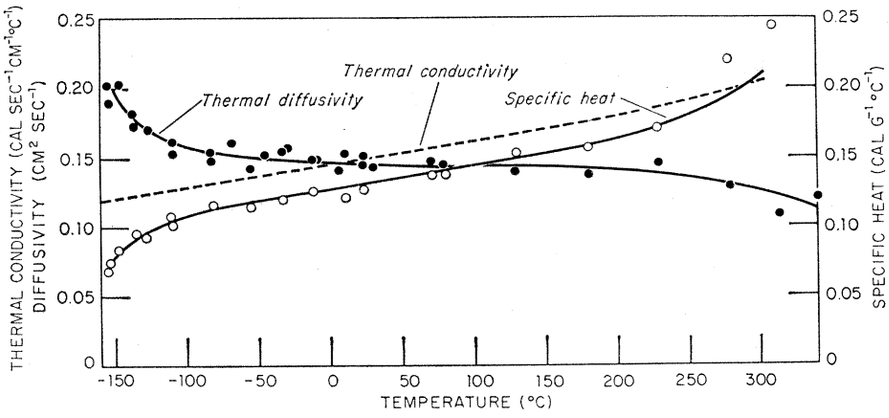

Fig. 2. Graph of experimental results for specific heat and thermal diffusivity. Dotted curve is calculated thermal conductivity.

The specimen used in this work was cut from one of the many pieces of the Canyon Diablo meteorite fall. This iron is an octahedrite which is a two-phase alloy, composed of low nickel kamacite and high nickel taenite. A square sample was cut measuring 2 cm on a side by 1 mm thick. It was ground on a surface grinder, the specimen being held with a magnetic chuck, until both sides were flat and parallel. The density of the specimen, from careful measurements of its dimensions and weight, was found to be 7.81 at room temperature. Examination of the specimen under the microscope did not reveal the presence of inclusions of troilite, schreibersite, or cohenite.

This thin section of meteoritic iron is presumably in its original state, that is, as it was before entering the earth's atmosphere. This is evidenced by the fact that the specimen was cut well below the surface, as well as by the appearance of the Neumann lines in the kamacite phase. There was no sign of granulation, characteristic of kamacite after it has been heated, either naturally or artificially. Structural changes occur in these meteorites when heated above approximately 400°C, and since none was observed, we assume that this specimen was thick enough so that aerodynamic heating did not penetrate to the depth from which the specimen was cut. If it were heated to this depth, then it must have been for a short time only, since such changes are both time and temperature dependent.

The photomicrograph shown in Fig. 1 is of the etched surface of the specimen used in these measurements taken after all temperature cycling was completed. The temperature was raised and lowered from 300° to −180°C several times during the course of the experiments, a range of temperature which we assumed would not cause any structural alterations to take place in the alloy.

However, it can be seen that the plessite field is thoroughly granulated, hence it may be concluded that some changes did take place over this temperature range. There is some evidence of incipient changes in the taenite phase as shown by patches of slight granulation brought out by the etching which is not clear in the picture. Despite these slight structural changes, the Neumann lines retain their original sharpness, indicating that no gross changes have occurred in the kamacite phase.

The results for thermal diffusivity and heat capacity and the calculated thermal conductivity as a function of temperature is shown in Fig. 2. Little weight is attached to the last two points in the specific heat curve, because at the higher temperatures carbon blacks tend to flake so that the absorption of the radiant energy by the specimen may not have been equal to that of the standard.

The results reported here on the thermal properties of meteoritic iron from −150° to 300°C are believed to represent those existing in the material prior to its encounter with the earth.

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30 October 1962

Tuberculin Hypersensitivity: Studies with Radioactive Antigen and Mononuclear Cells

Abstract. *The type and fate of mononuclear cells of guinea pigs hypersensitive to tuberculin were studied by means of purified protein derivative labeled with I^{125} and mononuclear cells labeled with tritiated thymidine. Purified protein derivative labeled with I^{125} was taken up in vitro by lymphocytes and neutrophils from animals that were either sensitive or nonsensitive to tuberculin, but it was bound more frequently by the cells of sensitive animals. Passive transfer of tuberculin hypersensitivity by means of lymphocytes labeled with tritiated thymidine indicated that significant numbers of radioactive cells migrated to the site where the skin was tested with purified protein derivative only when the test was made immediately after transfusion. Although skin reactions from tests made with purified protein derivative 24 hours after transfusion were comparable to those from tests made immediately, the number of labeled cells at the sites of the later tests was not consistently larger than it was in controls (Histoplasmin reactions). Thus transfused tuberculin-sensitive cells are neither always attracted to the sites of the test with purified protein derivative nor are they required in large numbers at the site for a positive reaction to develop.*

Neither specific identification of the cells reacting with tuberculin antigens nor clarification of their role in delayed cutaneous hypersensitivity has been achieved. In 1932 Rich and Lewis (*1*) showed that tuberculin was toxic for cells from tuberculin-sensitive animals. Favour (*2*) then reported that these cells were neutrophils in the guinea pig and small lymphocytes in the mouse and guinea pig. It was later noted that the affinity of leukocytes for tuberculin did not depend on an active tuberculous infection but was naturally present in species capable of showing a tuberculin reaction (*3*). Turk (*4*) found a greater uptake of purified protein derivative (PPD) labeled with I^{131} by sensitive lymphocytes but observed no qualitative difference in reacting cells from sensitive and nonsensitive animals.

Since 1945 when Chase (*5*) demonstrated that cutaneous tuberculin hypersensitivity could be passively transferred by lymphoid cells, there has been disagreement about the fate and func-

tion of the transfused cells. Waksman and Matoltsy (*6*) reported that transfused sensitive cells react directly with the antigen at the site of cutaneous testing, but there have been other conflicting reports as to whether transfused lymphocytes even reach the skin (*7*). Najarian and Feldman (*8*) recently published a thorough study on the fate of transfused, tuberculin-sensitive lymphoid cells labeled with tritiated thymidine and concluded that sensitive cells were specifically attracted to sites of tuberculin tests. Hamilton and Chase (*9*) also noted labeled cells at sites of positive skin tests, but thought it unlikely that these represented a selection of special cells.

These problems suggested two purposes for the present radioautographic study: first to report further on cell types and reactions which occur when labeled PPD is incubated with cells from either tuberculin-sensitive or nonsensitive guinea pigs, and second, to follow the early fate and role of tuberculin-sensitive lymphocytes in passively transferred hypersensitivity. Radioautographic techniques previously described (*10*) were followed. The PPD (*11*) was labeled with I^{125} (*12*) according to the method of Helmkamp (*13*), and lymphoid cells were labeled with H^3-thymidine in vivo (*14*). The advantages of these isotopes in facilitating high resolution radioautographs have been reported before (*10, 15*).

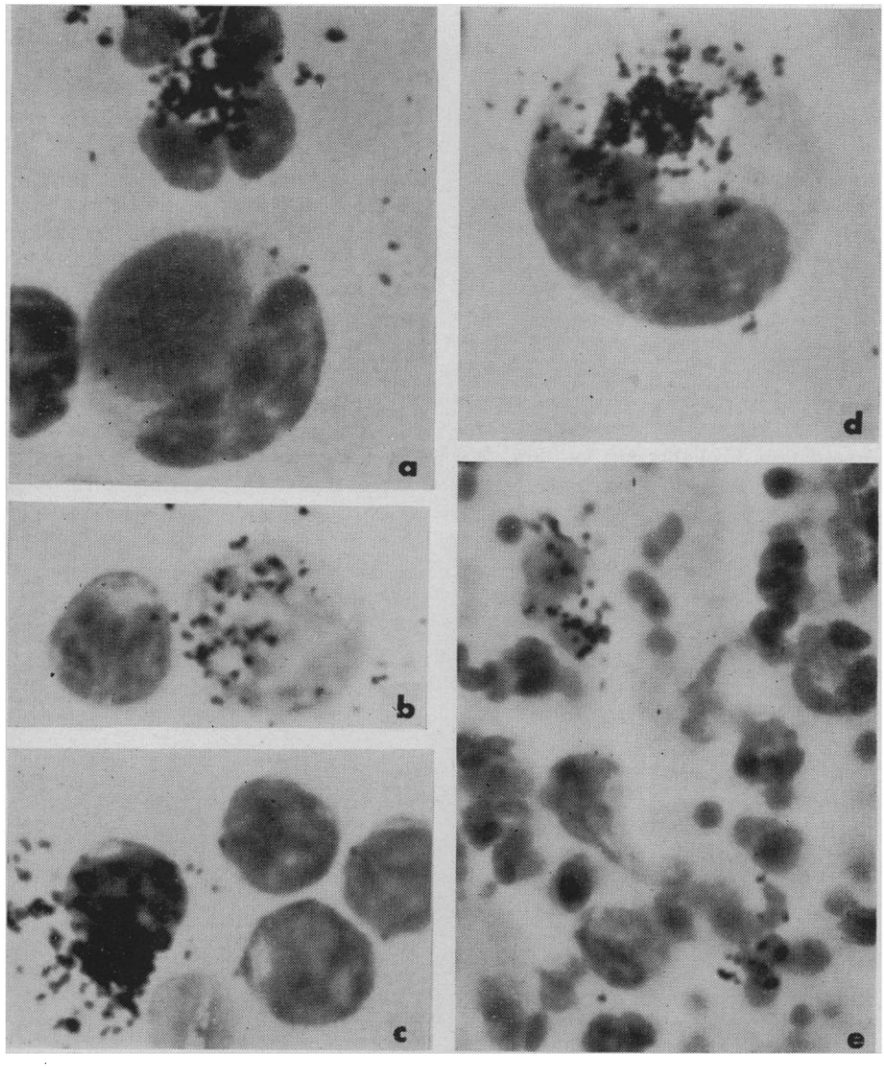

Fig. 1. Radioautographs of tissue smears and one skin section, × 1600. (*a*) Radioactive neutrophil from a suspension of tuberculin-sensitive spleen and lymph node cells after 20-minute incubation with PPD–I^{125}. Note also the mononuclear cell containing a large unlabeled Kurloff body. (*b*) Radioactive medium lymphocyte from a suspension of sensitive cells after incubation with PPD–I^{125} for 20 minutes. In contrast to Fig. 1*c* this cell appears to have actually incorporated the labeled antigen. (*c*) Radioactive small lymphocyte from a suspension of nonsensitive cells incubated 20 minutes with PPD–I^{125}. The pattern of the radioactivity suggests that the antigen has been largely adsorbed rather than incorporated. (*d*) Radioactive mononuclear cell from a suspension of sensitive cells incubated 20 minutes with PPD–I^{125}. The radioactive antigen is largely confined to a cytoplasmic vacuole. (*e*) Section showing inflammation and radioactive cells which developed after 24 hours in the ear (site of PPD test) of guinea pig No. 8 which received H^3-thymidine-labeled lymphocytes from a tuberculin-sensitive animal immediately before test was applied.

Four adult albino guinea pigs (375 to 800 g) were sensitized with multiple subcutaneous injections of live BCG vaccine (grown on Sauton's media and emulsified in Freund's adjuvant before injection). After reacting positively to a PPD skin test with an erythema at least 1 cm in diameter with central blanching and induration, two of the guinea pigs were used as cell donors for labeling studies in vitro and two as donors for labeling studies in vivo.

The teased cells of pooled thymus, spleen, and mesenteric lymph nodes from either sensitive or nonsensitive animals were used for experiments in vitro. To 1-ml portions of approximately 10^7 cells suspended in Hanks solution containing serum, 0.5 ml PPD labeled with I^{125} (0.15 mg PPD) was added. The suspended cells were incubated at 38°C for either 20 or 60 minutes, washed four times in Hanks solution, and suspended in serum. Smears were prepared from this suspension and used for radioautographs which were exposed 4 to 8 days.

To determine the results of passive transfer, one nonsensitive and two tuberculin-sensitive guinea pigs were injected intraperitoneally with 0.5 μc of H^3-thymidine per gram of body weight every 12 hours for 4 days. On the fifth day the spleens and mesenteric lymph nodes of the sensitive and nonsensitive animals, respectively, were pooled and teased in Hanks solution that contained serum. Approximately 1.4×10^8 nucleated cells (of which 16 percent were labeled) were injected intravenously into each recipient. Four animals received sensitive and two received nonsensitive cells. Immediately after this transfusion one-half of all the recipients were tested intradermally in the right ear with PPD (0.1 ml of a solution containing 0.1 mg/ml) and in the left ear with Histoplasmin (0.1 ml, three times normal concentration). Identical skin tests were performed on the remaining recipients 24 hours after

Table 1. Cutaneous reaction and labeled cells in skin and mesenteric node of guinea pigs skin tested immediately or 24 hours after receiving radioactive lymphocytes from tuberculin-sensitive or nonsensitive guinea pigs. The number of labeled cells per square millimeter was determined by counting all labeled cells in two or more sections and dividing by the total area of the sections calculated from planimetric tracings.

Animal	Cells received	Time of skin test after transfusion	Gross reaction after 24 hr		Labeled cells (No./mm²)		
			PPD-ear	Histo-ear	PPD-ear	Histo-ear	Mesenteric node
8	Sensitive	Immediate	+3	0	4.07	0.28	1.63
9	Sensitive	24 hr	+2	0	0.70	0.00	1.56
10	Sensitive	Immediate	+3	+1	2.72	0.61	1.48
11	Sensitive	24 hr	+3	+1	0.29	0.51	1.43
12	Nonsensitive	Immediate	+1	+1	0.39	0.12	1.21
13	Nonsensitive	24 hr	+1	0	0.00	0.00	1.75

transfusion. All skin test sites were examined periodically during the 24-hour period after antigen administration, the recipients were then sacrificed and histologic sections of the ear, thymus, spleen, liver, and mesenteric lymph nodes were prepared for radioautography. Radioautographs were exposed for 3, 5, and 7 weeks.

After incubating PPD labeled with I^{125} with cells from the spleen and lymph node, the labeled antigen was taken up by several types of cells from both sensitive and nonsensitive animals. Neutrophils (Fig. 1*a*), eosinophils, and medium and small lymphocytes (Fig. 1, *b* and *c*) from either experimental or control animals became labeled readily. More lymphocytes were labeled when sensitive cells were used. The labeled antigen frequently seemed to be only adsorbed on nonsensitive lymphocytes (Fig. 1*c*) whereas it seemed to be actually incorporated in the sensitive cells (Fig. 1*b*). Definite cytoplasmic ingestion of the antigen was seen in one type of mononuclear cell obtained from sensitive animals only (Fig. 1*d*). This type of cell, which measured from 10 to 20 μ in diameter and had an oval or indented nucleus, sometimes contained a few and sometimes many eosinophilic granules in the cytoplasm. Labeled antigen appeared in single or multiple vacuoles adjacent to the cytoplasmic granules. Kurloff bodies (Fig. 1*a*) were more frequently observed in cells from sensitive rather than nonsensitive animals, but these inclusions showed no radioactivity. From the above characteristics it was possible to attain an accuracy of 80 percent in separating sensitive from nonsensitive preparations in a coded, mixed series of smears.

To determine whether the binding of PPD to nonsensitive cells was entirely a nonspecific phenomenon, another protein, ovalbumin, was labeled with I^{125} and was tested in vitro with spleen and lymph-node cells from nonsensitive animals. From the result of the radioautographs the neutrophils took up ovalbumin as readily as they took up PPD, but lymphocytes combined with the ovalbumin much less frequently. Thus it appears that lymphocytes play an important and relatively specific role in the initial acceptance of tuberculin antigens. Both labeled ovalbumin and PPD were toxic to neutrophils, these cells degenerated rapidly after ingestion of these proteins. Death among lymphocytes which were labeled with the I^{125} was not commonly observed within the duration (1 hour) of the experiments.

Skin tests performed immediately following transfusion of sensitive cells yielded many more labeled cells (mostly lymphocytes, but some neutrophils) at the sites of the PPD than at the sites of Histoplasmin tests (Table 1, animals 8 and 10). This agreed with the report by Najarian and Feldman (*8*) and seemed to indicate a specific attraction of sensitive cells to the test site. If, however, skin tests were delayed 24 hours after transfusion, strikingly fewer labeled cells appeared even though gross and microscopic evidences of inflammation, similar to those resulting from tests immediately after transfusion, remained. Najarian and Feldman noted that fewer labeled cells were evoked when tests were delayed 3 or 4 days but reported that inflammation also was decreased and apparently was parallel to the number of labeled cells in the skin. They thought that labeled cells and inflammation were decreased because the transfused cells had gradually been lost from the lymphoid tissues of the recipient and, therefore, were not available for migration to the skin. Although entirely justified by the data from their tests which were delayed 3 or 4 days, these conclusions are not supported by the results of the present tests, made only 1 day after transfusion. Counts of labeled cells in tests made 1 day after transfusion show that there is neither an invariable parallelism between inflammation and the number of cells present in the skin, nor between the number in the skin and in lymphoid tissues (Table 1). Furthermore, sites of delayed PPD tests, although inflamed, did not consistently contain more labeled cells than the sites of relatively noninflamed Histoplasmin tests (Table 1, animals 9 and 11). There we have two observations which indicate that there is now obligate specific attraction of sensitive cells to cutaneous test sites.

Skin tests of control animals which received nonsensitive cells produced little inflammation and few labeled cells. As in transfers with sensitive cells, however, more labeled cells were present at the test site at 1 than at 24 hours (Table 1, animals 12 and 13). Thus, the number of isotopically labeled cells that appear in a transferred tuberculin reaction depends upon the number available to be attracted to the site of the skin test. A few hours after transfusion sensitized cells were neither specifically attracted to test sites nor were they present in large numbers in the developing skin reaction. Admittedly slight differences in experimental method and the small number of animals may have contributed to the variation between the present results and those inferred from a projection of Najarian and Feldman's to the particular circumstance of skin tests applied 24 hours after transfusion. However, the results of the present delayed tests are consistent and agree well with those from a larger series reported recently by Turk (*16, 17*).

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Electroencephalographic Changes during and after 14 Days of Perceptual Deprivation

Abstract. *A progressive decrease in frequencies in the alpha range was observed during 14-day exposure to unpatterned light and white noise. The electroencephalographic records were still abnormal 1 week later, and long-lasting motivational losses were observed.*

For several years researchers at the University of Manitoba have been studying the behavioral effects of 7-day periods of perceptual deprivation (*1*). Recently it was decided to extend the period to 2 weeks. It was considered important to obtain, first, some data on the electrical activity of the brain during isolation, particularly in the second week, as well as data for some days after termination of the period of isolation. Our report covers electroencephalographic changes in three individuals subjected to perceptual deprivation for this prolonged period.

Although there is a voluminous literature on the effects of sensory and perceptual deprivation, data on the electroencephalographic changes are almost nonexistent (*2*). In one of the McGill studies (*3*), electroencephalographic tracings for six subjects who were isolated from 3 to 6 days showed a progressive decrease in frequencies for the occipital lobe. Tracings taken 3½ hours after isolation still showed some signs of abnormality. Electroencephalographic records taken in the Manitoba studies also revealed a decrease in frequencies for the occipital lobe after 7 days of perceptual deprivation. These records showed that frequencies had not returned to normal 3 to 4 hours later (*4*). Finally, we have data on one subject isolated for 10 days. In this case the electroencephalographic activity was still abnormal 1 week after the end of isolation. It is of interest to note that this subject experienced a severe and long-lasting motivational loss (*1*). In the light of these meager but provocative findings, further research seemed warranted.

Three male subjects—two senior undergraduate students 21 and 22 years old, respectively, and a university professor 36 years old (subject C in Table 1)—were placed at different times in a dome-shaped isolation chamber for a period of 14 days. Toilet facilities, a food chamber, and an air-conditioning unit were provided within the chamber, making it unnecessary for the subject to leave for any reason during the isolation period. The only piece of furniture was an air mattress. Entrance to the chamber was through a double trapdoor in the floor, which also served as a food chamber. The behavior of the subjects was monitored at all times by means of an intercommunication and closed-circuit television system. The subject, wearing polo pajamas, lay on a mattress. He wore a pair of translucent goggles which reduced the level of ambient illumination from 90 to 20 ft-ca (under the goggles). He also wore a pair of special gloves to minimize tactual stimulation, and a set of earmuffs through which white noise, somewhat above the threshold of hearing, was constantly presented. He was not permitted to sing, hum, or engage in any other vocal activity. He was allowed to move about but not to exercise. Conversation over the intercommunication system was kept to a bare minimum; it occurred on the rare occasion when the subject did not adhere to certain restrictions, such as those against singing or humming. No psychological tests of any type were administered during the period of isolation. Each student was paid $300 for participating in the experiment. The professor was not paid. His incentive was scientific curiosity. There were no failures: the first three candidates selected successfully endured the prolonged isolation.

Electroencephalographic tracings were taken by an Offner type T, eight-channel machine. Records were taken before isolation and then during isolation at 7, 10, 12, and 14 days. At these specified times the electroencephalographer entered the chamber and attached a set of needle electrodes to the subject's skull. The entire intrusion lasted approximately 30 minutes. During this interval the white noise was shut off, but the subject continued to wear the goggles. Follow-up records were taken at 3 hours and at 1, 2, and 7 days after the end of the isolation period. In order to obtain a quantitative measure of the electroencephalographic changes, two types of analyses were

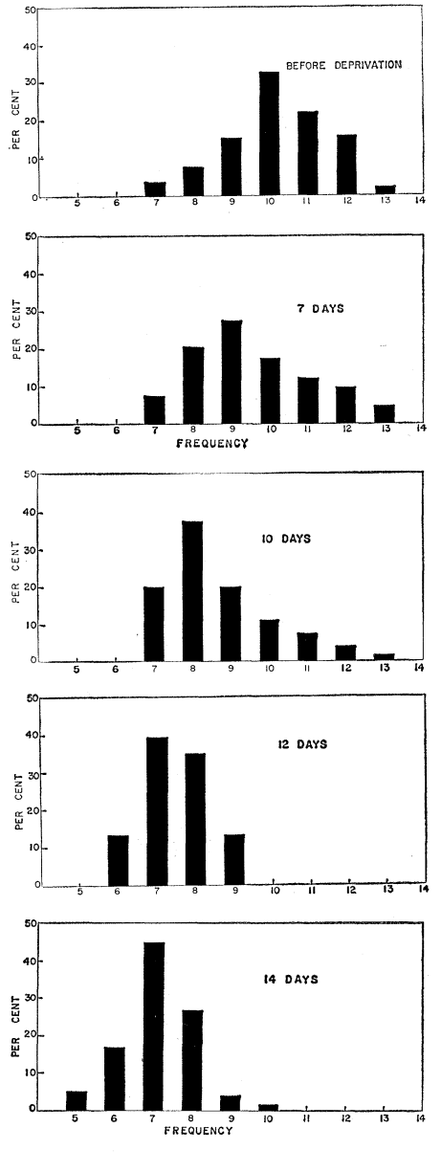

Fig. 1. Frequency spectrum for subject A before perceptual deprivation and at 7, 10, 12, and 14 days after the beginning of deprivation. The ordinate shows the percentage of time that waves of various frequencies appear in the occipital lobe tracings during a 300-second period.