made contact with plastic and rubber materials on chromatographic paper and examining the spots under ultraviolet light (3660 Å); and (ii) examining all the above-named liquids in quartz cuvettes under ultraviolet light (3660 Å). All the above-named solvents and solutions when stored in all-glass containers (controls), including glass-redistilled water autoclaved for 20 minutes at 15 lb/in.^{2}, did not show any ultraviolet fluorescent material (*4*). The results described here, as well as those of other workers, show that plastic and rubber laboratory apparatus usually considered chemically stable actually release undesirable substances under relatively mild conditions (*5*).

Though these fluorescent contaminants described here are not lethal to the growth of excised lemon fruit tissue in vitro, they may possibly exert adverse effects upon tissue growth. In addition, these contaminants make chromatographic analysis of nutrient solution for fluorescent material released from growing lemon fruit tissue more difficult. Consequently, all solutions of reagents, as well as glass-redistilled water, that I use for preparing nutrient solution are prepared in and handled with all-glass apparatus, and I grow excised lemon fruit tissue in all-glass vessels.

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1. H. A. Kordan, *Advan. Front. Plant Sci.* **10**, 59 (1965).
2. Bakelite screw caps that have been subjected to repeated autoclaving still release this fluorescent material during autoclaving. When autoclaved on vials without any liquid contents, there is enough residual moisture resulting from steam condensation to contaminate the vials with this ultraviolet fluorescent material from the Bakelite caps. Glass-redistilled water also extracts ultraviolet-fluorescent material from Bakelite caps with or without liners within 24 hours at room temperature. Water conditioning or water treatment is a standard procedure used by steam plants to prevent corrosion of the steampipes. The steam plant at the University of California, Los Angeles, treats water used for steam production with a strongly alkaline morpholine solution (formula 485, Garratt-Callahan Co.) to inhibit steampipe corrosion. This morpholine solution fluoresces and contaminates materials autoclaved with steam from the steam plant. Therefore, I autoclaved all material in an autoclave that was equipped with a self-contained steam generator.
3. In a personal communication from Rhoades Rubber Corp., Easthampton, Mass., the following substances used in compounding black rubber stoppers were listed as possible fluorescent materials extracted with alcohols: (i) naphthenic oil; and (ii) 2-mercaptobenzothiazal tetramethyl thiuram monosulfide. Pure latex-type (pharmaceutical grade) rubber stoppers were kindly furnished by Rhoades Rubber Corporation.
4. The alcohols listed in this study were reagent grade chemicals factory-packaged in brown glass bottles sealed with Bakelite screw caps. All these solvents showed a slight fluorescence under ultraviolet light (3660 Å), possibly resulting from the contact of these solvents with the Bakelite caps. Consequently, all the alcohols used in this study were redistilled and stored in all-glass containers.
5. J. W. DeWitt, *Science* **126**, 840 (1957); I. A. Boyd and C. L. Pathak, *Scot. Med. J.* **9**, 345 (1964); J. H. Hubschman and R. A. Engel, *Nature* **205**, 1029 (1965); J. Autian, *Am. J. Hosp. Pharm.* **18**, 329 (1961); M. J. Nicolaides and J. Autian, *Hospitals* **35**, 63 (1961); W. L. Guess and J. Autian, *Am. J. Hosp. Pharm.* **21**, 261 (1964); S. A. Rosenbluth, G. R. Weddington, W. L. Guess, J. Autian, *J Pharm. Sci.* **54**, 156 (1965). See also the extensive literature of J. Autian *et al.*, Drug-Plastic Research and Toxicology Laboratories, College of Pharmacy, University of Texas, Austin.
6. Supported by American Cancer Society grant E-293.
2 August 1965

Fatty-Tissue Changes in Rats with Acclimatization to Altitude

Abstract. *Adipose tissue in the adult white rat changes in both quantity and histologic characteristics during a 5-week period of acclimatization to a simulated altitude of 4350 meters at 26°C. These findings are descriptive and do not at present permit conclusions as to the mechanisms involved.*

Acclimatization to high altitude is a complex process that necessitates the adaptation of many tissues. There are no data regarding possible changes in adipose tissue during exposure in vivo to altitude, but species in which brown fat is found seem to have a greater capacity to survive under lower pressures of oxygen (*1*) than do species with less brown fat. A hypothesis that possession of brown fat contributes to tolerance of hypoxia might gain support if an increase in the quantity and distribution of brown fat could be demonstrated when animals are chronically exposed to reduced ambient oxygen in a thermoneutral environment.

Eight male, adult, Sprague-Dawley rats (286 to 304 g) were exposed, one per cage, to a simulated altitude of 4350 m for 5 weeks at 26°C (*2*); three of them did not survive. Another group of seven rats (284 to 307 g) was maintained as controls for 5 weeks at sea level at 36°C. All animals were then killed with Nembutal; brown fat from the superior cervical, interscapular, subscapular, pericardial, and axillary regions and white fat from the inguinal region were extirpated and

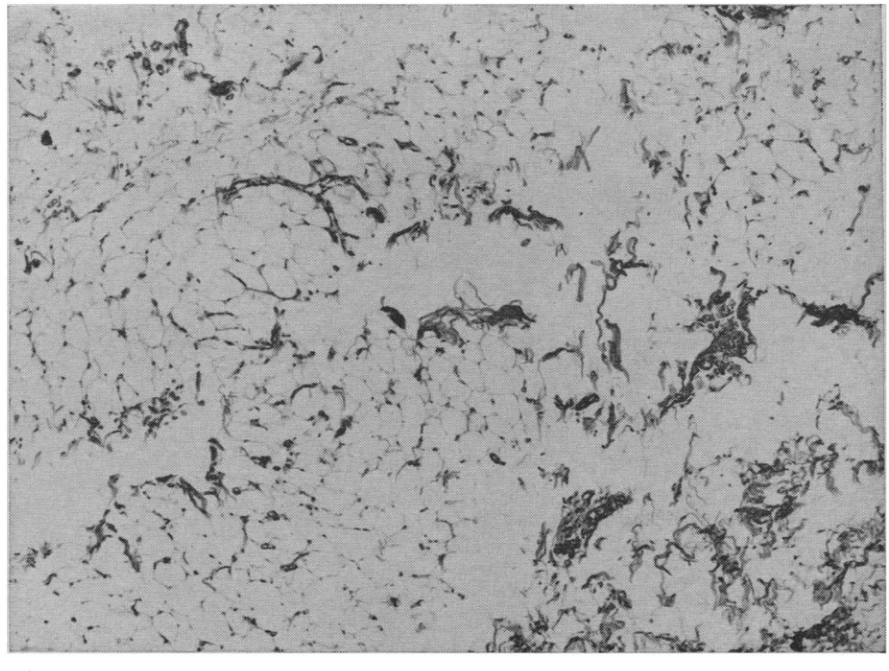

Fig. 1. Normal white fat from the inguinal fat pad of a rat kept at sea level; hematoxylin-eosin stain (× 100).

promptly weighed (wet) on a Voland-Universal balance. The data were statistically analyzed (Student t-test).

The mean absolute weight of the pooled, well-defined, brown-fat pads tended to be greater (Table 1) in the rats acclimated to 4350 m (0.500 g) than that in the controls (0.344 g), but the increase is not statistically significant ($p<.10$). Indeed, not all the pads showed the same degree of change, although, in general, distribution of the pads in the high-altitude animals showed overlaying onto adjoining areas, with spreading primarily along the blood vessels, which were dilated. However, this expansion of brown adipose tissue in the high-altitude animals becomes highly significant ($p<.001$) when it is related to mean body weights at the time of autopsy. The increase is magnified in part by the difference in final body weights; the rats exposed to altitude lost 11 percent of their weight, while the controls gained 36 percent. Loss of weight by the former was associated with a marked reduction in the total amount of white adipose tissue. Since conditions (such as starvation) that lead to a loss of body weight also cause a loss of brown fat as well as of white fat (*3*), retention of brown fat under the conditions of our experiment may add functional significance to this finding.

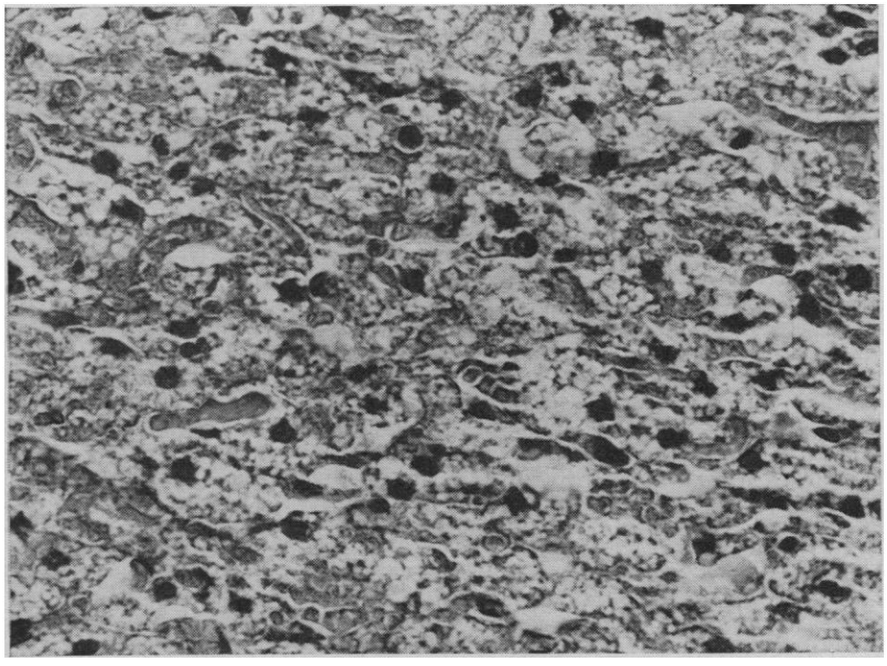

Fig. 2. Normal brown fat from the interscapular fat pad of a rat kept at sea level; hematoxylin-eosin stain. Note foamy cytoplasm and confluency of cells (× 633).

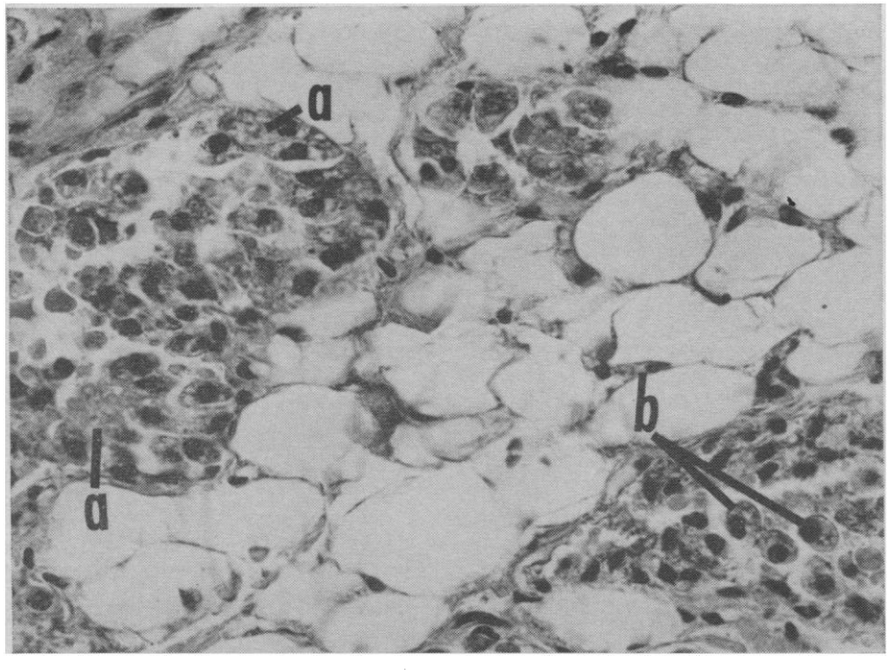

Fig. 3. Inguinal fat pad from a rat acclimated to 4350 m; hematoxylin-eosin stain. Note cell confluence, with foamy cytoplasm, in some areas (*a*), and cells with well-defined boundaries in others (*b*) (× 633).

Of special interest was what appeared to be the encroachment of new cells of brown adipose tissue into the white fat of the inguinal pad of experimental animals. Here the heaviest accumulation of new fat was centered well within the pad and radiated outward along the vessels. No such encroachment occurred in the controls. However, white adipose tissue that has lost large amounts of fat frequently resembles brown fat in gross appearance because of the relative predominance of blood vessels and protoplasm. To settle the question of whether or not the "increase" in inguinal brown fat in the experimental animals was caused by such changes, histological sections were prepared from the newly developed brown fat in the inguinal pad and compared with normal white (Fig. 1) and brown (Fig. 2) fatty tissues.

Examination of the inguinal pads from the experimental animals (Fig. 3) indicated an estimated 80-percent replacement of white-fat cells by different cells. The latter were suggestive of being proliferating, immature cells on the basis of a high nucleocytoplasmic ratio, highly basophilic nuclei, and dense cytoplasm. The tissue resembled brown fat in its partial lobulation, central oval nuclei, granulated cytoplasm and, in some sites, confluence of cells containing foamy cytoplasm. On the other hand, the new inguinal tissue was somewhat dissimilar to brown fat because of the presence of distinct cytoplasmic boundaries, the relative absence of foamy cytoplasm, and, in general, a more deeply staining cytoplasm.

The new cells may represent very immature or developing brown-fat tissue, since (i) structural characteristics typical of brown fat are recognizable, (ii) lipoblastic activity leading to development of white-fat cells is unlikely in an area of measurable loss of white fat, and (iii) brown-fat pads elsewhere were maintained or increased. These

Table 1. Weights of brown (pooled from five bodily regions) and white (inguinal) fatty tissues of rats after 5 weeks at sea level or at 4350 m (simulated); prime figures are followed by standard errors. Percentage changes in body weight appear in parentheses.

Brown fat		White fat, absolute (g)
Absolute (g)	Relative to body wt. (%)	
A, *Seven rats at sea level (+35.8±4.2)*		
0.344±.041	0.085±.008	4.055±.286
B, *Five rats at 4350 m (−10.9±3.4)*		
0.500±.070	0.192±.022	2.250±.359
Difference, B–A (−46.6±5.3)		
+0.155±.081	+0.107±.023	−1.805±.459
Probability, Student t-test (<.001)		
<.10	<.001	<.001

data thus suggest that brown fat may be an important factor in acclimatization of the rat to high altitude.

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Lymphocytes of Small Mammals: Spontaneous Transformation in Culture to Blastoids

Abstract. *Small lymphocytes from rabbit and guinea pig, grown in tissue culture in the absence of phytohemagglutinin, can transform spontaneously into cytologically immature cells that are indistinguishable morphologically from blast cells. The transformation occurs in 38 to 75 percent of peripheral lymphocytes and 55 to 75 percent of splenic lymphocytes on the 5th day of culture. The altered cells can synthesize RNA and DNA and show mitotic division; synthesis of DNA occurs after the 5th day of culture, whereas RNA is synthesized throughout the duration of culture.*

Despite participation of lymphocytes in many immunologic processes and their apparent role as the precursors of antibody-forming cells, their potentials and the specific factors that may control their differentiation and function have not been fully elucidated. Several substances, particularly phytohemagglutinin (PHA), can transform human peripheral lymphocytes, when grown in tissue culture, into large, immature blastoid cells (*1*). In the presence of PHA, 70 to 90 percent of the lymphocytes are morphologically immature even after being cultured for 72 hours, whereas cells grown without PHA are essentially unchanged.

While culturing human peripheral lymphocytes in the absence of PHA, I observed a striking alteration in both size and cytological characteristics in some of the cells on the fifth day of culture: between 5 and 40 percent of the cells were transformed, apparently spontaneously, into blast-like cells and were indistinguishable from those stimulated with PHA. Lest the presence of polymorphonuclear leukocytes in the lymphocyte culture inoculum be a possible stimulatory factor, the original plasma-leukocyte suspension was purified by allowing the polymorphs to adhere to glass prior to culture.

Four of the 14 donors had a history of allergy to penicillin; their cells were cultured without addition of antibiotics. They were in a group of nine donors in whom a spontaneous transformation of 5 to 16 percent of lymphocytes occurred, whereas the highest rates of transformation (19, 26, 29, 34, and 40 percent) were in donors without a history of allergy to drugs. This observation led us to investigate further the potential of mammalian lymphocytes for spontaneous transformation to blastoids and to study the metabolic function of such cells as determined by their ability to synthesize DNA and RNA. We have now shown that small lymphocytes from either rabbit or guinea pig can transform spontaneously into large blastoid cells capable of synthesis of RNA and DNA and of mitotic division. This cytological differentiation occurs in both peripheral and splenic lymphocytes and is evident in about 38 to 75 percent of cells on the fifth day of culture.

Blood obtained from nonimmunized rabbits by cardiac puncture was collected in syringes containing heparin. The erythrocytes were sedimented by adding one-half volume of dextran of high molecular weight to the whole blood and incubating the mixture for 20 minutes at 37°C. The supernatant plasma, rich in leukocytes, was then

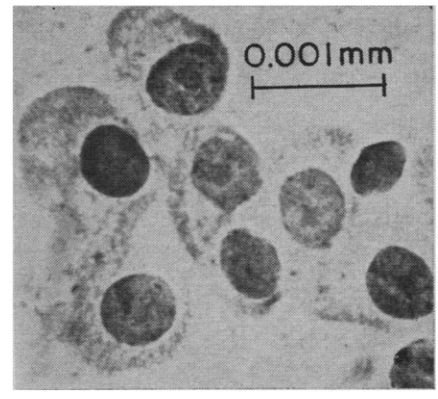

Fig. 1. Phase-contrast micrograph of rabbit peripheral lymphocytes grown in tissue culture for 5 days without phytohemagglutinin. The lymphocytes have transformed spontaneously into large blastoid cells possessing abundant cytoplasm, delicate nuclear chromatin, and prominent nucleoli.

aspirated and centrifuged at 800 rev/min for 5 minutes. The cells were washed twice with Eagle's minimal essential medium and the total lymphocytes were counted. Replicate cultures, each containing 3×10^6 lymphocytes in 4 ml of medium (minimal essential medium, modified for suspension culture, supplemented with 20 percent fetal calf serum and 1 percent L-glutamine), were incubated for 5 days at 37°C.

Phytohemagglutinin type M (0.1 ml) was added to some of the tubes at the beginning of culture, and these cells were grown for 72 hours. Duplicate cultures were harvested within one hour, and then two or more cell cultures from each animal were harvested 3 and 5 days later. The cells were examined by phase-contrast microscopy after being fixed in a mixture of absolute methanol and glacial acetic acid (3:1) and being stained with 0.5 percent acetic-orcein. The cells were also examined by light microscopy after being stained with Jenner-Giemsa; however, nuclear detail was considerably enhanced by phase-contrast microscopy, which was used for most of the counts. One thousand cells on each slide were counted, and the percentages of typical small lymphocytes and of altered lymphocytes were determined.

The cytological criteria for transformation were based both on the size of the cells and on the appearance of the nuclear chromatin. Thus cells with prominent nucleoli and a pale-staining, delicately reticulated nuclear chromatin were considered transformed even if they were of about the same size as