Table 1. Fatty acids and cholesterol content of extrahepatic and liver in 18 adrenotropic tumor-bearing mice (ATO), 13 adrenalectomized tumor-bearing mice (Adrex-T), and 22 controls.

	Mice	Carcass lipid content			Liver lipid content			
		Fatty acids		Cholesterol (mg)		Fatty acids		Cholesterol (mg)
Type	Wt. (g)	Wt. (g)	(%)		Wt. (g)	Wt. (mg)	(%)	
Controls	27.1±2.9	2.10±0.63	7.78±2.48	54.8± 9.71	1.54±0.04	0.049±0.019	3.80±1.43	4.88±0.94
Adrex-T	27.8±2.86	1.77±0.49	6.21±1.27	52.9±12.40	1.56±0.15	0.063±0.026	4.04±1.18	4.73±1.18
ATO	26.8±2.52	4.25±1.12	17.30±5.16	70.2±10.79	1.82±0.25	0.107±0.069	6.13±2.66	6.72±1.58

diture among the three groups. The ATO mice under study did not show glucosuria, although they showed some polyuria. The fact that weight remained stationary in the ATO mice despite the manifestly positive energy balance can be interpreted when body composition (Table 1) is considered.

Body composition was determined on a total of 54 animals: 18 ATO mice, 14 Adrex-T mice, and 22 controls. The tumors were small—2 to 5 mm in diameter. Cholesterol and fatty acids were determined by standard methods. The cholesterol digitonide precipitation results were checked by Sperry-Webb (5) determination on the acetone-ethanol extract. Results in Table 1 show that the ATO mice, despite their normal weight, were effectively obese because they contained twice as much extrahepatic fat as controls and 3 times as much as the Adrex-T animals. Liver fat is similarly elevated as are both carcass and liver cholesterol. All differences concerning carcass fat and cholesterol are highly significant, with Student's t values between 5 and 10. Differences in liver fat are significant ($p < 0.001$ between ATO and controls, $p < 0.01$ between ATO and Adrex-T). The difference in liver cholesterol between ATO and controls is significant ($p < 0.01$).

In previous studies (reviewed by Mayer, 6), a distinction has been established between "metabolic" and "regulatory" obesities. In metabolic obesity, which is exemplified in mice by the obese-hyperglycemic syndrome, lipogenesis from acetate is increased over the control values even when both obese and control animals are submitted to restricted feeding or fasting. Reduction in weight to the normal figure does not restore normal body composition. Such characteristics are not seen in regulatory obesity, which is exemplified in mice by goldthioglucose and hypothalamic obesities. The ATO animals obviously fulfill one of the criteria of metabolic obesity: considerably elevated fat content even when the body weight is normal. The considerably increased body cholesterol content, which is also seen in the obese hyperglycemic syndrome but not in regulatory forms of obesity, is also suggestive. Studies of C^{14}-carboxy-labeled acetate incorporation show very significantly increased lipogenesis and cholesterologenesis in fasting, as well as nonfasting, conditions and confirm the metabolic nature of this new type of obesity (7).

Mice bearing adrenotropic tumors provide an additional illustration of the difference between *overweight* and *obesity*. They appear to constitute an interesting example of metabolic obesity. Finally, they are a useful tool in the study of the mode of action of corticosteroids.

JEAN MAYER
CLAIRE ZOMZELY
J. FURTH

Department of Nutrition, Harvard School of Public Health, and *Childrens Cancer Research Foundation, Boston, Massachusetts*

References and Notes

1. J. Furth, E. L. Gadsden, A. C. Upton, *Proc. Soc. Exptl. Biol. Med.* 84, 253 (1953).
2. This work was supported in part by grants No. A-49 and C-2259 from the National Institutes of Health and by the Sugar Research Foundation, the Kellogg Company, and the Nutrition Foundation, Inc. We gratefully acknowledge the excellent assistance of Ann J. Stoops and Evelyn Gadsden.
3. J. Mayer, *Science* 117, 504 (1953).
4. J. Mayer *et al.*, *Endocrinology* 50, 318 (1952).
5. W. M. Sperry and M. Webb, *J. Biol. Chem.* 187, 97 (1950).
6. J. Mayer, *Voeding* 16, 62 (1955); *Nutrition Abstr. and Revs.*, in press.
7. A report of these studies is in preparation.

22 August 1955

Summer Jobs for High-School Students

What is the attitude of high-school students toward careers in science? In a recent survey, Melvin Barnes, assistant superintendent of the Oklahoma City public schools, asked 100 high-school juniors why more students did not take courses in science and mathematics. Although I am only a junior in high school, I would like to give a brief account of this survey and then offer my own idea on how to improve the attitude of students.

One of the startling answers to Barnes' questions was "Einstein! Long hair and a sweat shirt." Other students answered by describing scientists as "squares" or "little old men with beards working in musty laboratories." The majority pictured mathematics and science courses as being dull. Also, some students stated that higher education in any scientific field was expensive, while the job opportunities after graduation were poor. Barnes concluded from his survey that there was a need for better vocational counseling and hinted that better teaching methods might make science subjects seem less difficult.

Since I am not a member of the teaching profession, I am unable to comment on Barnes' conclusions. However, I would like to offer a suggestion of my own. My idea is to place the task of encouraging students to choose a scientific career in the hands of all members of the scientific field. In many high schools there are programs by means of which students are permitted to gain "on the job" experience in the commercial fields. Why are not summer jobs offered to interested high-school students as laboratory aides or the like? Such students are just as capable of carrying out laboratory procedures as clerking in a store or stocking shelves. The point that I am trying to bring out is that one summer of actual work in the field of science is a greater encouragement to decide upon a scientific career than a year of constant lecturing on the subject by a teacher. This sort of program also inspires the student to apply for scholarships if he cannot afford higher education. It is certainly beneficial to the student in the way of experience that will be useful to him in college.

JAMES G. BUSSE
Cotter High School, Winona, Minnesota

12 January 1956

Magnetic Techniques for in vitro Isolation of Leucocytes

Over a period of time, this laboratory has undertaken studies on various techniques for the isolation, *in vitro*, of leucocytes in blood. Since relatively little has been published concerning the applicability of certain techniques investigated here, a brief preliminary note is presented to summarize our experience with these methods.

The usual approach to the isolation of white cells from human blood has been to increase the sedimentation rate of the erythrocytes by means of the fibrinogen technique (1). However, it has been found that this technique suffers from several shortcomings. First among these was the observation that the white cell fraction so obtained is appreciably contaminated with 30 to 60 percent erythrocytes. Moreover, it was found that the bovine fibrinogen technique is limited in its applicability because it does not produce any observable effect on the sedimentation rate of freshly collected and citrated bovine blood or sheep blood.

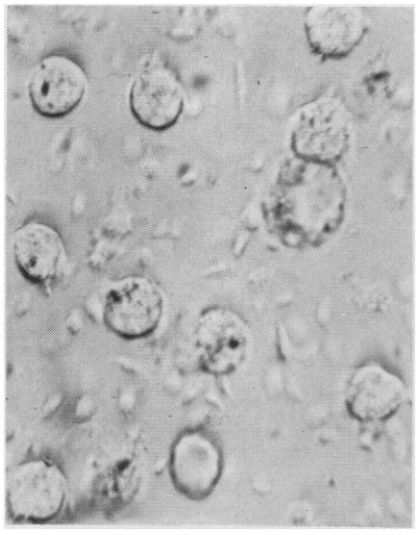

Fig. 1. Microphotograph showing leucocytes with phagocytized iron that appears as dark spots within the leucocytes. Starch particles are visible in the background.

In an attempt to develop a more efficient and generally applicable technique, a series of studies was undertaken in an effort to utilize certain characteristic properties of the several classes of cells that are found in blood. Experiments were performed in an attempt to utilize the paramagnetic properties of hemoglobin as a method of selectively removing erythrocytes. Since the ferrous iron in the porphyrin ring of hemoglobin has four uncoupled electrons in the third orbitals (*2, 3*) these should contribute a magnetic moment μ, given by

$$\mu = \sqrt{n(n+2)} = 4.90 \text{ Bohr magnetons},$$

where n is the number of uncoupled electrons. This paramagnetism is lost, however, when the ferrous iron complexes with certain molecules such as oxygen, cyanide, and others (*4*). Under these circumstances, the uncoupled electrons form hybridized orbitals of the d_2sp_3 and related types. Although the paramagnetic properties of hemoglobin should result in a relatively weak attraction of the erythrocytes into a magnetic field, it was felt that the approach warranted at least some preliminary study.

Venous blood was drawn directly from patients and passed through an ion-exchange column of Amberlite 112 in the sodium form. In this manner, calcium was removed without the addition of complexing agents, which could conceivably interfere with the paramagnetic properties of the ferrous iron. The blood was then collected in a 12-inch, 5-mm-bore glass tube, deoxygenated under vacuum, and finally sealed under a nitrogen atmosphere. The blood was then subjected to a highly inhomogeneous field of about 20,000-gauss maximum intensity at the center (*5*). The sedimentation of the blood so treated was compared with that of similar samples that had not been subjected to the magnetic field. Despite the known paramagnetic properties of hemoglobin, no readily observable difference in the two sedimentation rates was apparent. This failure is, however, not surprising since, as previously mentioned, it can be shown that the potential energy effect for the paramagnetic ion is quite small; in addition, there is the countervailing action of the diamagnetic cell constituents and blood proteins.

Because of the difficulties inherent in these techniques, it was decided to attempt isolation of the cells by employing the phagocytic properties of certain of the leucocytes *in vitro*. A similar approach *in vivo* has been reported by Rous and Beard (*6*) and others (*7*). In the studies reported here, an attempt was made to induce *in vitro* phagocytosis of iron granules. Phagocytosis of iron granules in the experiments was ascertained by observing the response of the phagocytes to an applied magnetic field. When ingestion of an iron particle had occurred, the ingested particle appeared as a dark spot (1 to 3 μ in diameter) within the leucocyte, and the cell underwent a distinct movement when a magnetic probe was brought in proximity to the microscope slide. The experiments were conducted in plastic- or silicon-lined glass test tubes in an incubator that was maintained at 37°C. Gentle stirring was provided by a special rotary rack that revolved the test tubes about the long axis at a slow rate of speed. Initial experiments in which iron powder (*8*) was added directly to citrated human blood resulted in little if any uptake over a period of 2 hours. Microscopic observation revealed that the direct addition of iron to the blood samples resulted in the formation of large clumps of iron that were unable to undergo phagocytosis because of their large size.

In an attempt to produce a more stable iron suspension and in order to increase the phagocytotic propensities of the leucocytes, a special iron-starch preparation was finally developed that gave satisfactory results. This preparation was made by dissolving 10 g of solubilized starch in 200 ml of isotonic sodium chloride, then adding 20 g of powdered iron. The suspension was then brought to 80°C for 30 minutes and was finally filtered through a layer of gauze in order to remove some of the larger aggregates. The iron particles in the preparation so obtained showed little tendency to settle out even after the preparation had been left standing for several hours. Microscopic observations indicated that the iron particles had become coated with starch. Attempts were made from time to time to grind the iron particles in order to increase the 1-to-3-μ portion. However, it was found that grinding the powder in a number of devices such as an automatic agate mortar, ball mill, and in recently designed colloidal mills (*9*) did not materially affect the size distribution of the powdered iron.

When the preparation was used, appreciable phagocytosis occurred. A typical experiment was conducted by adding 5 ml of the iron starch preparation to 20 ml of freshly drawn and heparinized or citrated blood in a plastic test tube. The sample was incubated at 37°C while it was undergoing slow rotation. Samples of the blood were examined at half-hour intervals over a period of 2 hours. Under the conditions described, appreciable phagocytosis occurred as is shown in Fig. 1. The iron is visible as small dark spots within the phagocytes. A number of starch granules are visible in the photograph. In a number of instances, it could be shown, by rotating the cells with a magnetic probe, that the iron was not completely phagocytized but rather remained adhering to the cell membrane. Extension of these experiments to white cell preparations that were obtained by means of the fibrinogen technique showed that such cells also phagocytized iron under the conditions described. Indeed, in several instances the fibrinogen-treated cells showed greater iron phagocytosis than cells not so treated.

This *in vitro* phagocytosis of iron provides a convenient method for the magnetic isolation of phagocytes. A number of promising arrangements are now under investigation in this laboratory. One such consists of transferring the incubated blood samples to a paraffin-coated petri dish mounted over an electromagnet. With the field on, the phagocytes are firmly held to the bottom of the dish while the blood protein and erythrocytes can be removed by gently flushing the system with isotonic saline. When the flushing operation has been completed, the magnetic field is cut and the phagocytes are collected by flushing with the saline.

SUMNER LEVINE
East Orange Veterans Hospital, East Orange, New Jersey

References and Notes

1. R. Baer, F. Serri, P. Kirman, *J. Invest. Dermatol.* 19, 217 (1952).
2. L. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci. U.S.* 22, 195 (1936).
3. R. Pauling, W. B. Whitney, W. A. Felsing, *J. Am. Chem. Soc.* 59, 633 (1937).
4. R. Pauling and C. D. Coryell, *Proc. Natl. Acad. Sci. U.S.* 22, 210 (1936).
5. I am indebted to the Metallurgical Laboratory of Bell Telephone Laboratories, Summit, N.J., for making available the electromagnet that was used in these studies.
6. P. Rous and J. W. Beard, *J. Exptl. Med.* 59, 577 (1934).
7. S. St. George, M. Friedman, S. O. Byers, *Science* 120, 463 (1954).
8. Fisher Scientific, hydrogen-reduced.
9. I wish to thank the Admiral Tool and Dye Company of New York for supplying a colloidal mill.

22 August 1955