its separation from the metabolite (*3*).

In case No. 1, death was attributed to acute barbiturate intoxication and positional asphyxia. A half-filled bottle of phenobarbital tablets was found near the body.

The entire sample of urine (390 ml) was buffered at *pH* 7 and exhaustively extracted with ether. The ether extracts were combined, washed with 0.2*N* HCl, dried with powdered Na_2SO_4, and shaken with 0.05*N* NaOH until all the barbiturate was extracted. The ultraviolet absorbencies were recorded at *p*H 9.5 and *p*H 2. The barbiturate was then reextracted with fresh ether from acid solution, the volume was reduced, and the ether solution was applied to 6 sheets of paper for chromatography (*3*).

For a more controlled preliminary study, two 2-ml samples of the original urine were extracted in parallel by the afore-mentioned procedure. In order to locate and study the zones after chromatography, we used the following procedure: initial scanning of one strip by densitometry (*4*) *pinpointed the* R_f values; the strip was eluted section by section with 0.05*N* NaOH, and the ultraviolet absorption spectra were recorded to determine which of the substances present possessed spectra characteristic of the 5,5-disubstituted barbiturates. At the same time, quantitation was accomplished. The other strip was split vertically in two, and the silver acetate and potassium permanganate tests were applied (*3*).

Two barbiturates were found. One was located at R_f 0.50 and was presumably phenobarbital. It gave a positive result with the silver acetate reagent and a negative result with potassium permanganate. The other barbiturate was located at R_f 0.29 and gave positive results with both reagents. The results obtained when known and comparable amounts of both phenobarbital and *p*-hydroxyphenobarbital (*5*) were carried through the same procedure coincided in every respect.

The phenobarbital and its metabolite were removed from the 6 sheets of paper. Each substance was recrystallized from hot water. Mixed melting-point determinations with the known pure compounds proved the presence of phenobarbital and *p*-hydroxyphenobarbital in the urine.

The concentration of the metabolite was calculated from the absorptivity of the *p*-hydroxyphenobarbital. The concentration of phenobarbital was 5.8 mg/100 ml of urine. The concentration of *p*-hydroxyphenobarbital was 11 mg/100 ml.

The 390 ml of urine that had been extracted was then hydrolyzed acording to Butler's method (*1*); after hydrolysis, the procedure was continued as outlined here.

The concentration of *p*-hydroxyphenobarbital was found to be 9.2 mg/100 ml of hydrolyzed urine. The compound was recrystallized and mixed melting-point determinations confirmed the identification.

A barbiturate concentration of 7.2 mg/100 ml of blood was found by ultraviolet spectrophotometry (*2*). The results of a paper chromatographic study as outlined for urine showed that the only barbiturate detectable in extracts from 25 ml of blood was phenobarbital.

A 30-g sample of homogenized liver was analyzed in the same manner as blood. A barbiturate concentration of 12 mg/100 g of liver was found. The chromatographic study showed that only phenobarbital was present in the liver extracts.

Only 25-ml of fluid stomach contents were available. This sample was analyzed by the same procedure that was used for blood and found to be negative for barbiturate.

In case No. 2, a bottle known to hold 75 1½ grain tablets of phenobarbital was found empty in a coat pocket of the deceased. A study of the urine and blood disclosed results similar to those obtained in case No. 1.

In the first case presented, 46 percent of the *p*-hydroxyphenobarbital was conjugated; in the second, 20 percent was in the conjugated form.

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15 August 1955

Body Composition and Energetics in Obesity Induced in Mice by Adrenotropic Tumors

Some of the characteristics of a transplantable pituitary adrenocorticotropic tumor induced in mice that had been exposed to ionizing radiation have been described (*1*). The salient changes produced in the recipients are enlargement of the adrenals (and of no other endocrine organ,) leukopenia (notably lymphocytopenia), atrophy of the thymus and the spleen, polyuria rarely with glycosuria and hyperglycemia, obesity, and sensitivity to infection that usually kills the host while the tumor is still small. Some tumor-bearing mice reach weights in the neighborhood of 45 to 50 g as compared with a limit of about 30 g for controls of the same strain (LAF_1). In the course of successive passages, the "overweight" component of obesity became less marked, although obesity (as defined by a very much increased fat content) was still always present. This is particularly evident in the experiments presented here, where the mice, bearing small grafted tumors, had a normal weight but more than twice the normal fat content. In other words, they are "obese"—that is, grossly "overfat"—as determined by body composition.

This report (*2*) compares the energetics and body compositions of adrenocorticotropic tumor-bearing mice (ATO) with those of normal controls and of adrenalectomized tumor-bearing animals (Adrex-T) of similar age. It was noted earlier that obesity along with other secondary changes rapidly disappears if the adrenals of the tumor-bearing mice are removed. Therefore, the inclusion of this last type of animal provides a test of the effect of the tumor per se without the overproduction of adrenal hormones.

A total of 47 mice of the LAF_1 strain were used in the study of energetics. Sixteen of these mice had been grafted with tumors 10 weeks previously; at the time of the study, they weighed 27.0 ± 2.4 g. Fourteen animals of the same strain and age were grafted at the same time and immediately adrenalectomized; they weighed 27.4 ± 2.1 g when studied. The 17 control animals weighed 27.0 ± 2.5 g. Spontaneous activity was determined over a 3-week period using rotary (squirrel) cages as previously described (*3*); the groups were rotated among 16 cages so that each animal spent a total of 7 days under measurement. Food (ground Purina chow) intake was recorded for each animal during that period. Basal oxygen consumptions were determined as previously described (*4*). Results point to a positive energy balance in the ATO animals: spontaneous activity, expressed in number of revolutions per day, was found to be 6024 ± 3003 for the controls, 4336 ± 1997 for the Adrex-T mice, and 4555 ± 2439 for the ATO mice. The difference is significant ($p < 0.002$) between control and ATO animals, but it is not significant between the two groups of tumor-bearing mice. Food intakes were 4.8 ± 1.0 g/day for the controls, 5.1 ± 0.7 g/day for the Adrex-T mice, and 5.8 ± 0.9 g/day for the ATO mice. The difference between the controls and the ATO mice is highly significant ($p < 0.001$); between the Adrex-T and ATO mice, it is significant ($p < 0.002$). There was no difference in basal oxygen expen-

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.

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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.

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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.