together with those on the catalase oxidation of Hg^0 to Hg^{2+}, indicate the presence of an oxidation-reduction cycle for mercury in mammalian cells, the oxidation step being in the peroxisomes and the reduction step in the cytoplasm. Since most organomercury compounds degrade to Hg^{2+} in mammalian tissue, Hg^0 should be present in tissues after exposure to virtually all forms of organic or inorganic mercury. Hg^0 may play an important general role in the physiological distribution of the metal after administration of all forms of mercury because of its lipid solubility and high diffusibility (*14*).

The elucidation of mercury oxidation and reduction pathways shows that our concepts which restrict the role of metallic cations to enzyme inhibition must be revised. Mercury acts not only as an enzyme inhibitor but as substrate for oxidation-reduction reactions, much like organic xenobiotics (*15*). Reactions in which a metal serves as substrate, such as oxidation-reduction [Hg, Cr (*16*), As (*17*), and Ni (*18*)] and alkylation-dealkylation [Hg (*19*), As (*20*), and Sn (*21*)] will probably be an important focus for future heavy metals research.

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6. Modified 15-ml Warburg flasks were used, and continuous air flow (100 ml per minute) above the tissue preparation swept volatilized mercury onto a Hopcalite (Mine Safety Appliances) adsorbent which was counted for radioactivity. Recoveries of known amounts of $^{203}Hg^0$ generated in these flasks were 98.8 ± 5.1 percent [mean ± standard deviation (S.D.)]
7. Actual results in picograms of Hg per 30 minutes [means ± standard error (S.E.), $N = 5$] were: 8.1 ± 2.0, 10.9 ± 1.4, 33.0 ± 1.7*, 46.4 ± 1.9, 52.2 ± 2.8, 49.3 ± 2.5 for 0, 2.5, 25, 50, 75, and 100 mg of fresh liver, respectively (*statistically significant difference from control values, $P < .05$, Student's *t*-test). All flasks contained standard amounts of heat-deactivated tissue, mercury-cysteine complex, and ethanol (613 mg/dl, initially). Control flasks received buffer only.
8. This protocol was used despite the depression of maximal mercury volatilization in the presence of deactivated tissue because the background (residual) volatilization was low, the experimental reproducibility high, and the method facilitated comparison of specific MVA.
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11. AT, an irreversible inhibitor of catalase activity [T. R. Tephly, G. J. Mannering, R. E. Parkes, *J. Pharmacol. Exp. Ther.* **134**, 77 (1961)], was dissolved in saline (250 mg of AT per milliliter). Fresh liver homogenates were incubated in 90 m*M* AT for 1 hour at 4°C before MVA tests. Control homogenates received only saline. The mercury-cysteine complex and either ethanol or saline were added after the incubation period. Ethanol (where added) was initially 123 mg/dl (27 m*M*). Final tissue concentration in all flasks was 3.3 percent (weight per volume). Amounts of Hg (in picograms) volatilized in 30 minutes (means ± S.E., $N = 5$) were: controls, 19.8 ± 3.9; AT, 98.2 ± 4.6*, ethanol, 109.3 ± 3.7*; AT ± ethanol, 155.6 ± 7.3** (*significantly different from control values, $P < .05$, Student's *t*-test; **significantly different from values obtained with either AT or ethanol alone, $P < .05$, Student's *t*-test).
12. Ethanol-derived acetaldehyde has been reported to decrease reduced glutathione in isolated hepatocytes [J. Vina *et al.*, *Biochem. J.* **188**, 549 (1980)]. Conceivably, decreased concentrations of glutathione could contribute to increased mercury volatilization in our ethanol experiments by decreasing thiol sites available for mercury binding. Although the exact relation of glutathione to MVA is not known, we believe its role, if any, is a minor one because cysteine, which readily forms adducts with acetaldehyde [A. I. Cederbaum and E. Rubin, *Biochem. Pharmacol.* **23**, 2179 (1976)] was added in excess to our incubation media and increased MVA was observed with AT alone.
13. Two mice were injected intraperitoneally with 0.5 mg of Hg per kilogram as ^{203}Hg-labeled $HgCl_2$ and 48 hours later checked for mercury exhalation (*4*). Animals were then killed and 5 percent (weight per volume) homogenates made from pooled mouse livers or kidneys. Mercury concentrations were determined from samples of each homogenate, and the remainder was tested for MVA, with or without ethanol. Initial mercury concentrations in the incubation media, as determined by radioactivity counting, were 24 ng for liver and 488 ng for kidneys [natural background total (organic plus inorganic) mercury concentrations in untreated CBA/J mice were < 1.5 ng/ml and ≤ 3.3 ng/ml for 5 percent liver and kidney homogenates, respectively]; initial ethanol concentrations were either 31 (low) or 613 mg/dl (high). Final tissue concentration in all flasks was 3.2 percent. Results of 30-minute tests in picograms of Hg (means of duplicate or triplicate results) were: control (no ethanol) liver, < 1; control (no ethanol) kidney, 20; low ethanol liver, ~ 3.5; low ethanol kidney, 140; high ethanol liver, 11.8; high ethanol kidney, 230.
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Brown Adipose Tissue: Thermic Response Increased by a Single Low Protein, High Carbohydrate Meal

Abstract. *The weight of interscapular brown fat in the rat and its rate of respiration increased in response to a single meal. These data suggest that brown adipose tissue plays a role in the thermic effect of meals and that diet-induced thermogenesis may reflect the summation of the thermic effects of single meals during prolonged overeating.*

Brown adipose tissue in the rat increases its thermogenic activity in response to long-term overfeeding (*1*). Our data suggest that this same tissue may play a role in the thermic response to a single meal.

Increased oxygen uptake after a meal was first reported by Lavoisier in 1789 (*2*). Rubner (*3*) called this phenomenon the specific dynamic effect (SDE) and ascribed the elevation in heat production after a meal to energy wasted in the ensuing metabolic processes. The nature of the reactions leading to the production of SDE, however, is still not clear; and SDE has been regarded as an expression of energy lost in the process of digestion and absorption (*4*), amino acid oxidation and urea formation (*5*), protein synthesis (*6, 7*), and other metabolic conversions (*8*). The effect was found by some to be considerably greater after a protein meal than after a carbohydrate or a fat meal (*3, 9*), while others found it to be unrelated to the type of the macronutrient consumed (*7, 10, 11*). When mixed meals are offered, the total SDE is considerably smaller than the sum of SDE's produced when each macronutrient is provided separately (*10, 12, 13*). The organ primarily responsible for SDE has not been clearly identified, although the liver has been considered to play an important role (*14*).

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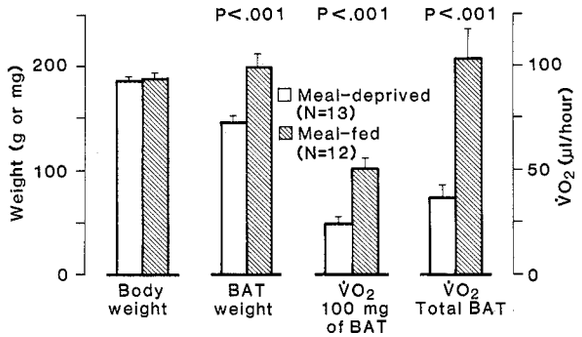

Fig. 1. Body weight, interscapular brown adipose tissue (*BAT*) weight, and rate of respiration ($\dot{V}O_2$). The respiratory rate is expressed as oxygen uptake per 100 mg per hour or per total tissue per hour. Data are means ± standard error. Statistical analysis was done by Student's *t*-test. The test meal contributed 39.4 ± 4.1 kcal, with protein contributing 8 percent, fat 23 percent, and carbohydrate 69 percent.

We examined the effect of a single meal on tissue weight and on the uptake of O_2 by brown fat. In addition, O_2 uptake by brown adipose tissue was compared to O_2 uptake by the liver. Twenty-five female Wistar rats (Simonsen), weighing 180 to 200 g at the start of the experiment, were used; they were caged individually and maintained at 24° ± 2°C, with lights on from 0700 to 1800.

During five consecutive days the rats had access to food between 0900 and 1100 and again between 1530 and 1730. This 5-day period was followed by 2 days of free access to food. The same cycle of scheduled feeding followed by free access to food was repeated during two consecutive weeks. After these 2 weeks the rats were put on their scheduled feeding plan for one additional day. On the experimental day, one group was allowed access to food for 2½ hours, while the other was deprived of food. All rats were decapitated during the second hour after the food was removed. The diet used in this study was either Purina Lab Chow (basal diet) or Purina Lab Chow supplemented with chocolate chip cookies and with 16 percent maltose as the only available drinking solution (cafeteria diet). The cafeteria diet was offered twice weekly in the morning only, and this diet also served as the test meal. The purpose of this feeding plan was to train the rats to eat larger meals, to accustom them to the test meal, and to minimize a state of nutrient depletion incurred by scheduled feeding. The composition of the test meal was based on previous findings that a high-carbohydrate, low-protein diet was highly thermogenic (*15*). Food intakes were recorded at the end of each meal, and body weights were recorded every 3 or 4 days and also before the morning meal on the day the animal was killed. Interscapular brown adipose tissue was dissected immediately after the death of each rat; the tissue was weighed, and 100 to 200 mg of the chopped tissue was used for the measurement of respiratory rate. Oxygen uptake was measured in a Gilson differential respirometer with a Krebs-Ringer bicarbonate buffer (containing 50 percent Ca^{2+}). After a 30-minute equilibration time, 10-minute recordings were made for 30 minutes. Linearity of respiration was evident in all cases.

Brown adipose tissue from the fed rats was 38 percent heavier than that from the meal-deprived rats (Fig. 1). The tissue from the fed rats exhibited a more than twofold increase in respiratory rate on a weight basis and an almost threefold increase for the total tissue in comparison with the tissue from the meal-deprived rats. All measurements were statistically significant ($P < .001$). In a second experiment, 24 additional rats were fed under a similar experimental protocol to obtain measurements of O_2 uptake in liver samples. There was no difference in respiratory rate of liver between the meal-fed and the meal-deprived groups. The respiration rate of liver samples from fed rats was 35.2 ± 2.1 μl of O_2 per 100 mg per hour and from nonfed rats was 37.1 ± 3.6. In the same study, the respiration rate of brown adipose tissue in the fed rats was more than double that in the nonfed rats (39.5 ± 6.7 compared to 91.4 ± 14.9 μl of O_2 for the total tissue per hour). Thus, if the liver contributes to SDE, its contribution is too small to be detected by our method. Although our data do not rule out the liver as a site for production of SDE, our observation of a significant enlargement as well as an increase in the metabolic activity of brown adipose tissue in vitro after a meal suggest that brown adipose tissue contributes to this phenomenon. The increase in respiratory rates of brown adipose tissue in vitro may reflect changes in stored energy of the tissue or the action of hormones produced in response to the meal, or both. Because our incubation medium did not provide modulating substances such as energy substrates, hormones, or neurotransmitters, our results in vitro should be considered as reflecting "endogenous" O_2 uptake and therefore cannot be quantitatively equated with rates of respiration in vivo.

The most outstanding characteristics of brown fat are its high rate of substrate oxidation and its consequent large capacity to produce and distribute heat. Oxidation of substrate by this tissue may serve as a homeostatic mechanism by which a diet unbalanced in its macronutrient composition can become more balanced by selective oxidation of one or more macronutrients present in excess (*16*). As has been repeatedly shown, a single meal balanced in its macronutrient composition is associated with a much lower level of SDE than that attained from each separate macronutrient or a disproportionate mixture of macronutrients (*10, 12, 13*). Evolution of heat by brown fat may serve a second possible homeostatic function, namely, the regulation of energy balance. Excess energy intake over periods of several weeks to several months may be dissipated as heat, and this phenomenon has been called dietary thermogenesis (*17*). Debate as to the magnitude and mechanism, and even the existence of this phenomenon, has been going on for about eight decades (*18, 19*). Dietary thermogenesis was reported in rats overfed a cafeteria diet for 20 days, and the suggestion was made that brown fat may be important in this mechanism (*1*). Our data showing that respiration in this tissue is greatly increased in response to a single meal suggests a common metabolic pathway for the two phenomena; dietary thermogenesis in the rat may be an extension of the SDE's of single meals. Support for this suggestion is based on previous findings that SDE is reduced in undernutrition (*3, 20*) whereas in undeprived subjects the size of SDE is dependent on the size of the meal consumed (*3, 18, 21*). Thus SDE and dietary thermogenesis may be one phenomenon.

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Fertilizability of Ova Ovulated and Recovered from Rabbit Ovaries Perfused in vitro

Abstract. *Ovaries removed from New Zealand White rabbits were perfused and exposed to gonadotropin in vitro. The ova ovulated in vitro (N = 56) were recovered and cultured and then transferred to the oviducts of six previously mated Dutch Belted hosts. Twelve of the resulting 36 offspring (33.3 percent) were white. In control matings between 12 Dutch Belted females (six randomly selected and the six hosts) and New Zealand White males, only one of 80 (1.2 percent) offspring was white. These data indicate that ova ovulated in vitro can be transferred to the oviduct of a host rabbit where they may be fertilized and after implantation may develop into viable embryos.*

Perfusion of the rabbit ovary in vitro has served as a valid model for studying mammalian ovulation. Serial observation of the final stages of follicle development and disruption facilitates assessment of the effects of various agents added to the perfusate which may have a direct ovarian action (*1–6*). The effluent can be serially sampled for determination of substances produced by the ovary during the ovulatory process (*7*). The stage of maturity of ova recovered immediately after ovulation in vitro can be correlated with the time interval from gonadotropin stimulation to the occurrence of ovulation (*8*). Ovulation in the rabbit occurs within 12 hours after the administration of human chorionic gonadotropin (hCG) or mating (*9*). In the study described here we determined that ova extruded from ovaries in vitro achieve the same degree of maturation after exposure to gonadotropins in vitro as they do in vivo and that such ova are capable of being fertilized in vivo.

Rabbits were isolated for a minimum of 3 weeks with controlled temperature and light and were given free access to a diet of Purina Rabbit Chow and water. Ovaries from sexually mature virgin New Zealand White (NZW) rabbits served as a source of ova. Sexually mature virgin Dutch Belted (DB) female rabbits were used as hosts for ova ovulated in vitro. The characteristic black fur of the DB rabbit serves as a marker to distinguish DB offspring of the host rabbit from those of the NZW rabbits which were used as ovum donors. Male NZW rabbits of proved fertility served as inseminators.

Both ovaries of untreated NZW female rabbits were removed after arterial cannulation at laparotomy and perfused individually according to the operative procedure and perfusion technique described previously (*1, 6, 8*). At the onset of perfusion 100 IU of hCG was added to the perfusion fluid of all ovaries. This dose promotes follicular development, follicular rupture, and ovum maturation consistently in this system (*7, 8*). Perfusion was carried out in a constant temperature room (37°C) maintained at 100 percent humidity. Ovaries were observed continually for follicle development and ovulation. Follicles that continued to grow throughout perfusion and achieved a diameter of greater than 1.5 mm were considered mature. Ovulation in vitro was characterized by disruption of the follicle wall and extrusion of the ovum surrounded by cumulus cells.

The ovulatory efficiency, defined as the percentage of mature follicles which rupture by 12 hours after hCG administration, of the 14 perfused ovaries used in this experiment was 75.5 ± 2.2 percent (mean ± standard error); the mean time of ovulation was 6.03 ± 0.29 hours after the addition of hCG. These data are consistent with previous observations (*8*).

Each ovum was aspirated by Pasteur pipette from the surface of the ovary immediately after ovulation. Ova were washed three times in Brackett's defined medium and cultured according to the method of Brackett *et al.* (*10*) until 12 hours had elapsed from the initial time of exposure to hCG in vitro. At 12 hours after hCG addition, ova were removed from the culture dish together with a small amount of culture medium for transfer to the host rabbit.

The time period selected for ovum culture after hCG treatment was based on an experiment in which 20 NZW ovaries were perfused as described above. Seventy-eight ova were cultured after ovulation in vitro until a total of 12 hours had elapsed from the time of hCG administration. All ovulations in vitro occur by 12 hours after exposure to hCG (*8*). Ova were microscopically examined for stage of maturity. Ova demonstrating cytolysis, necrosis, vacuolation, or loss of spherical shape were classified as degenerated. Of the ova treated in this fashion, 1.3 percent contained an intact germinal vesicle with no degeneration, 15.4 percent achieved metaphase 1 (germinal vesicle breakdown) with no degeneration and 51.3 percent were at metaphase 2 (first polar body extruded) and had not degenerated. The 12-hour interval from hCG administration to transfer was therefore selected to yield a high percentage of mature ova (metaphase-2 ova) to be transferred to the host rabbits for fertilization in vivo.

A DB female host was mated with an NZW buck at the same time as each ovarian perfusion was begun (Fig. 1). The vagina of the DB host was examined 8 hours after mating to confirm the presence of sperm. Twelve hours after mating, each host underwent a laparotomy in preparation for transfer of ova from the donor rabbit. Ova were transferred from the culture dish to both oviducts of the host by means of a glass pipette in which the flow was controlled by a screw mechanism to prevent leakage of fluid and loss of ova. The pipette was inserted into the ampulla of the oviduct and the ova, in approximately 20 μl of Brackett's medium, were gently deposited into each oviduct. The number of ova transferred in each experiment corresponded to the number of ova recovered from the donor

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