Reports

Adipose Tissue: Ability to Respond to Nerve Stimulation in vitro

Abstract. *Electrical stimulation of nerves to isolated rat and rabbit adipose tissue in vitro causes production of free fatty acids. Starvation increases the response. The response of white (epididymal) fat is prevented by sympathetic denervation. Direct evidence is provided showing that adipose tissue has the capacity to be an effector organ responsive to the nervous system.*

The ability of the nervous system to influence activity of adipose tissue is supported by the observations in the past reviewed by Wertheimer and Shapiro (*1*). The study of the effect of various autonomic blocking agents on the release of free fatty acids (FFA) into the blood has provided indirect evidence that the sympathetic nervous system exerts an important influence on the metabolism of adipose tissue (*2*). Numerous nerve fibers apparently supplying the cells of white fat and of brown fat have repeatedly been demonstrated since the early observations of Boeke (*3*) and Hausberger (*4*). This is a report of an in vitro technique whereby direct evidence of the ability of adipose tissue to respond to electrical stimulation of its nerve supply was obtained.

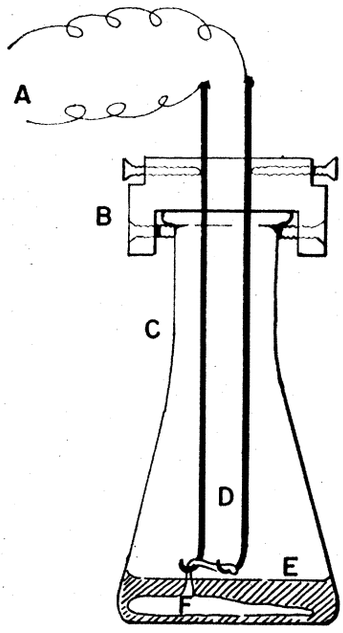

Fig. 1. Plastic unit (*B*) carrying chlorided bipolar silver electrodes (*D*) mounted on a 25-ml erlenmeyer flask (*C*), is shown in contact with pedicle containing nerves to isolated adipose tissue (*F*), immersed in medium (*E*). Wire leads (*A*) carry electrical stimulus.

In these experiments, summarized in part in previous communications (*5*), epididymal white adipose tissue of rats and rabbits (*6*) and the interscapular brown adipose tissue of rabbits were utilized. Nerves to the paired epididymal fat pads are contained in a long slender neurovascular pedicle passing through each inguinal canal. Nerves to the paired interscapular fat pads of rabbits are visible and can be isolated by dissection. The entire epididymal fat pad, or a small section (weighing 100 to 200 mg) of the brown fat pad, with the nerves attached, was removed after pentobarbitol anesthesia. The tissue was incubated at 37°C, while being agitated in a medium Krebs-Ringer phosphate buffer solution containing 5 percent bovine serum albumin (Armour, fraction V) adjusted to *p*H 7.4. The nerves were placed across stimulating electrodes mounted in a 25-ml erlenmeyer flask just above the level of the medium in which the attached fat was immersed (Fig. 1). Electrical stimulation was provided continuously for 2 hours with a 5-volt, 1.0-msec square wave pulse at a frequency of 50 per second. The FFA concentration was estimated, by the method of Dole (*7*), on aliquots of medium before and after incubation. At the end of the experiment the tissue was weighed wet and the production of FFA recorded as microequivalents per gram wet weight of tissue per hour (μeq/g hour). In all experiments either the other member of paired fat pads or other portions of the same pad served to provide control observations.

The production of FFA from one epididymal fat when compared to the other from the same animal was similar when each tissue was treated in an identical manner (Fig. 2).

Stimulation of white or brown fat always resulted in a significant increase in the production of FFA ($P < .001$). Starvation, known to increase the effect of epinephrine on white adipose tissue (*8*), also increased the response to stimulation. Stimulation of the epididymal fat pad from rats starved 24 hours resulted in an increase in FFA production of 0.57 (SE 0.04) μeq/g hour beyond that of the nonstimulated control (Fig. 2), while in fed rats the increase in FFA was 0.31 (SE 0.06) μeq/g hour. Dibenamine, an adrenergic blocking agent, in a concentration of 0.5 mg/ml of medium, decreased the response to stimulation of tissues from starved rats to 0.15 (SE 0.04) μeq/g hour beyond the nonstimulated controls, whereas sympathectomy (*9*), carried out 4 to 11 days before stimulation, completely prevented it (Fig. 2).

The response of the epididymal fat pad from rabbits was similar to that from rats. Electrical stimulation of nerves to isolated interscapular brown adipose tissue of rabbits resulted in an increase in the production of FFA greater than that for epididymal white adipose tissue. For 29 preparations of

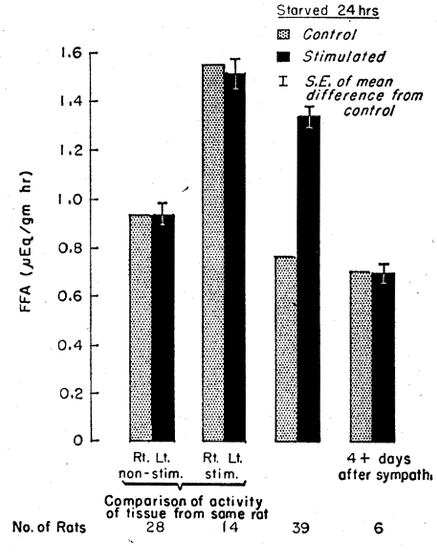

Fig. 2. The production of free fatty acids (FFA) by one epididymal fat pad, as compared to the other member (Rt. = right, Lt. = left) of the pair from the same rat is shown in the absence (nonstim.) and in the presence of electrical stimulation of each. The effect of stimulation compared to nonstimulated controls is shown. Four or more days after sympathectomy (*9*) stimulation was without effect.

brown adipose tissue removed from the interscapular brown fat pad of 21 rabbits there was an average increase of 2.099 (SE 0.45) μeq/g hour beyond that of nonstimulated controls.

These observations indicate that both white and brown adipose tissue respond to nerve stimulation. The response of white fat is dependent on nerves of sympathetic origin, since it is decreased by the presence of dibenamine and completely prevented by sympathectomy, timed to allow nerve degeneration.

The isolated nerve-fat preparation has been used to explore some additional factors which effect this neuroeffector system (*10*), and it should facilitate further investigation of this mechanism (*11*).

JAMES W. CORRELL
Department of Neurological Surgery, College of Physicians and Surgeons, Columbia University, New York

References and Notes

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3. J. Boeke, *Z. Mikroskop. Anat. Fortschr.* **33**, 276 (1933).
4. F. Hausberger, *ibid.* **36**, 231 (1934).
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6. Albino rats weighing 200 to 300 g and market-bought rabbits of mixed breeds, fed a stock diet and weighing between 2500 and 3500 g, were used.
7. V. P. Dole, *J. Clin. Invest.* **35**, 150 (1956).
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9. The lumbar sympathetic chain had been excised asceptically.
10. J. W. Correll, *Federation Proc.* **21**, 280 (1962).
11. Supported by the Paul Moore Fund and by grant B-3581, National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

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Tryptophan- and Indole-Excreting Bacterial Mutants

Abstract. *Prototrophic mutants of* Escherichia coli *capable of producing indole and tryptophan were obtained by selecting for resistance to antimetabolites. An anthranilic acid auxotroph grown under conditions which would derepress the tryptophan synthetase system would also accumulate substantial quantities of* L*-tryptophan.*

The usual method of obtaining microbial cultures which synthesize substantial amounts of amino acids is by screening, through trial and error, isolates from soil samples or random mutants from a known culture. Adelberg (*1*) has suggested a more rational method which is based upon the use of substances antagonistic to the desired metabolite.

An extension of such an approach is being applied in our laboratory to obtain bacterial mutants which can produce substantial quantities of extracellular L-tryptophan. *Escherichia coli* K-12 was subjected to ultraviolet (UV) radiation and then was plated on modified M-63 (*2*) agar containing 10 mg of 5-methyltryptophan and 2.0 g of glucose (instead of glycerol) per liter. Of the 5-methyltryptophan-resistant mutants which developed, several were constitutively derepressed with respect to the tryptophan synthetase system (*3*). The mutant with the greatest activity of tryptophan synthetase was treated again with ultraviolet radiation and plated on modified M-63 agar containing 300 mg of anthranilic acid per liter; this substance has been reported to inhibit the conversion of anthranilic ribonucleotide to indole-3-glycerol phosphate (*4*). Such treatment might select mutants with a synthesizing enzyme for indole-3-glycerol phosphate that has altered sensitivity to anthranilate or mutants more completely derepressed than the parent in some or all of the enzymes that produce tryptophan.

The resulting doubly resistant mutant cross-fed with an auxotroph of *E. coli* that responded to either indole or trytophan; it did not cross-feed with an auxotroph which responded only to tryptophan (*5*). Treatment of the doubly resistant mutant with ethylmethanesulfonate (EMS) (0.03 ml/2 ml of culture) produced mutants which would cross-feed with the tryptophan-responding auxotroph. When one of these EMS-produced mutants, chosen because of its large zone of cross-feeding, was grown while being shaken at 37°C on M-63 broth containing glycerol, an indole concentration of 300 mg/liter resulted after 20 hours of incubation. The final turbidity of the culture was 350 Klett units with a 640-mμ filter. Approximately 50 mg of tryptophan per liter were also present at this time (*6*). It is probable that the indole is derived from tryptophan by the action of the enzyme tryptophanase because the addition of serine had no effect on the accumulation of tryptophan. Although a similar procedure was applied to *Aerobacter aerogenes* in order to obtain derepressed mutants which lacked tryptophanase, and which would therefore accumulate only tryptophan, such efforts have not yet been successful.

When an auxotrophic mutant of *E. coli* unable to synthesize anthranilic acid (*E. coli* T3; *7*) was grown with growth-limiting amounts of anthranilic acid (3 mg/liter), thus causing derepression of the tryptophan synthetase system, and then was harvested by centrifugation and suspended (turbidity of 300 Klett units) in double-strength M-63 broth plus 1 g of anthranilic acid per liter, L-tryptophan accumulated; a peak concentration of 100 mg/liter was reached at 4 hours. Under these conditions, tryptophanase was induced and the tryptophan was degraded to indole, which accumulated to 300 mg/liter in 10 hours. If chloramphenicol (5 mg/liter) was added simultaneously with the excess anthranilic acid, synthesis of the induced enzyme tryptophanase was prevented, and tryptophan accumulation continued to a peak of 250 mg per liter in 24 hours. At this time less than 1 mg of indole per liter was present.

Previous workers have failed to detect such substantial tryptophan or indole excretion, even with constitutively derepressed or feedback-insensitive mutants. *Escherichia coli* 5 MTR-6 (*8*), a 5-methyltryptophan-resistant mutant insensitive to feedback inhibition, which excreted approximately 1 mg of indole per liter, was repressible, while Yanofsky (*9*) found that several strains of *E. coli* and hybrids of *Shigella* and *E. coli* which produced high levels of tryptophan synthetase excreted no tryptophan because of a partial block at some early step in the synthesis of tryptophan. Thus, it appears that in the case of prototrophs a single mutation either to constitutive deprepression or to insensitivity to feedback inhibition may not be sufficient to cause significant accumulation of the end product of the pathway. On the other hand, single-mutation auxotrophs blocked after the branch point of the pathway (*E. coli* T3) may readily accumulate the end product if supplied with limiting amounts of the precursor, thereby causing derepression; then a surplus of precursor is added under nongrowing conditions (*10*).

P. G. LIM
R. I. MATELES
Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge 39