it would be accessible to antibody (*11*). However, we have been able to detect LDH-X on the surface of maturing spermatozoa (*12*), and this could provide a basis for an inhibitory effect of antiserum to LDH-X on fertilization in vitro. The antiserum may have blocked sperm penetration by enhancing sperm aggregation and subsequently preventing sperm-egg interaction. Spermatozoal toxicity was unlikely because the antiserum used for the fertilization experiments in vitro was heated to inactivate complement, and the gamma globulin fractions lack some of the components of complement. Nevertheless, although the plasma membrane of sperm fuses with the plasma membrane of the egg and some LDH-X might be brought to the egg membrane by the sperm, there was no effect on development when the early embryo was exposed to high concentrations of antiserum to LDH-X (Tables 2 and 3), again indicating that the effect was on sperm and not on the egg or zygote.

ROBERT P. ERICKSON
Department of Pediatrics, University of California, San Francisco 94143
PETER C. HOPPE
Jackson Laboratory, Bar Harbor, Maine 04609
DANIEL TENNENBAUM
HORST SPIELMANN
CHARLES J. EPSTEIN
Department of Pediatrics, University of California, San Francisco 94143

References and Notes

1. E. Goldberg and J. Lerum, *Science* **176**, 686 (1972).
2. E. Goldberg, *ibid.* **181**, 458 (1973); J. E. Lerum and E. Goldberg, *Biol. Reprod.* **11**, 108 (1974).
3. E. Goldberg, *J. Biol. Chem.* **247**, 2044 (1972).
4. H. Spielmann, R. P. Erickson, C. J. Epstein, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **35**, 19 (1973).
5. ———, *Anal. Biochem.* **59**, 462 (1974).
6. D. Bennett and E. Boyse, *Nature* **246**, 308 (1973).
7. C. J. Epstein, E. A. Wegienka, C. W. Smith, *Biochem. Genet.* **3**, 271 (1969); W. K. Whitten, *Nature (Lond.)*, **179**, 1081 (1957); M. S. Golbus, P. A. Calarco, C. J. Epstein, *J. Exp. Zool.* **186**, 207 (1973).
8. A. C. Menge, A. Rosenberg, D. M. Burkons, *Proc. Soc. Exp. Biol. Med.* **145**, 371 (1974).
9. P. C. Hoppe and S. Pitts, *Biol. Reprod.* **8**, 420 (1973).
10. S. Auerbach and R. L. Brinster, *Exp. Cell Res.* **46**, 89 (1967); J. Rapola and O. Koskimies, *Science* **157**, 1311 (1967); R. L. Brinster, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **17**, 41 (1971); R. P. Erickson, H. Spielmann, F. Mangia, D. Tennenbaum, C. J. Epstein, *Proc. Int. Isozyme Conf. 3rd*, in press.
11. C. J. Epstein, L. Kowk, S. Smith, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **13**, 45 (1971).
12. R. P. Erickson, D. Friend, D. Tennenbaum, *Exp. Cell Res.*, in press.
13. Supported by grants from the Population Council, New York, and NIH grants HD-03132 and HD-04083, by NIH research contract HD-72-2740, and by a research career development award NICHHD (R.P.E.).

16 October 1974

Arsenic Tolerance in a Population of the Grass Andropogon scoparius Michx.

Abstract. *Samples of* Andropogon scoparius *Michx. collected on an arsenic mine exhibited a wide range of tolerance to arsenic in solution, whereas plants of the same species growing in uncontaminated soil showed no tolerance. Arsenic tolerance must be an evolved character under genetic control. Furthermore, the degree of tolerance is related to the amount of arsenic in which the plant was growing.*

The study of heavy metal tolerance in plants provides one of the best available examples of evolution in action (*1*). Mine populations of the common bent grass, *Agrostis tenuis* Sibth., have evolved edaphic races which exhibit tolerance to lead, zinc, nickel, or copper (*2*). Plant populations growing on an abandoned arsenic mine in Floyd County, Virginia, provide an ideal opportunity to study the effects of arsenic, which is not a heavy metal and which is not known to be an essential mineral nutrient. In fact, arsenic compounds are known to be toxic to higher plants (*3*). It has been reported that tolerance in man and some other mammals can be induced through gradual habituation (*4*). Therefore, the presence of several plant species on the Floyd County mine immediately raises the question of whether these populations belong to species inherently tolerant to arsenic or whether they have become habituated or have actually evolved a tolerance to arsenic.

Plants of *Andropogon scoparius* Michx., little bluestem, the dominant mine species, were collected along several transects at 5-meter intervals. Each plant was individually potted in normal soil and placed in a cold greenhouse. Control plants from two populations of *A. scoparius* found in uncontaminated soil were also tested. Samples of the control-Floyd population, C-F, were collected within a half a mile (1 mile = 1.6 kilometers) of the mine, while plants from the control-Montgomery population, C-M, came from 25 miles away. Using the method of Wilkins (*5*), we compared root growth of individual tillers in Na_2HAsO_4 solutions of various concentrations with root growth in the absence of arsenic, taking the length of the longest root. Testing was done in all-glass aquariums, and Pyrex or stainless steel stirrers were used to prevent concentration gradients and to ensure aeration. Arsenic concentrations chosen for this test were 1, 3, 5, 10, 25, and 50 ppm as elemental arsenic. We did not test at less than 1 ppm because of the difficulty of maintaining such low concentrations (Table 1).

Soil samples were collected from the root zone of each plant. After oven drying, each sample was analyzed for total arsenic content by means of x-ray emission spectrometry. The soil arsenic concentration in parts per million (dry weight) is shown for each plant in Table 1. Although the total amount of arsenic in the soil is not necessarily the amount available to the plant, it is an indication of the relative amounts potentially able to affect plant growth (*6*).

The results show that the mine population possesses a tolerance to 1 ppm arsenic which the control plants lack. As the concentration was increased, the mine plants showed a steady decrease in root growth. The mine population

Table 1. Root growth of *A. scoparius* Michx. in arsenic solution as a percentage of control growth.

Plant	Soil arsenic total (ppm)	Plants (No./m²)	Growth (%) in arsenic solutions at*					
			1 ppm	3 ppm	5 ppm	10 ppm	25 ppm	50 ppm
D-5	41,200	2	95	82	64	64	22	10
D-1	25,300	5	90	69	46	29	9	0
D-8	20,500	4	85	58	43	25	10	0
D-9	17,000	2	91	79	49	40	23	10
D-2	14,600	6	91	75	36	25	9	0
D-4	6,100	7	85	41	19	8	0	0
D-3	1,320	9	91	50	30	20	0	0
E-1	310	6	86	13	2	0	0	0
E-2	100	11	67	11	0	0	0	0
C-F	10		0	0	0	0	0	0
C-M	10		0	0	0	0	0	0

* Except for control populations, each figure represents the mean of five tillers from each plant. The figures given for the two control populations (C-F and C-M) represent the averages of five tillers from each of five plants.

exhibited a wide range of tolerance. Plants E-1 and E-2, which were found growing in the least soil arsenic, showed the least degree of tolerance, while D-5 and D-9 from highly arsenical soil continued growing in 50 ppm arsenic in solution.

These data suggest that the ability of *A. scoparius* to tolerate soils contaminated with arsenic is the result of a genetic change wrought by selection. If, as is likely, there is some genetic variation for arsenic tolerance inherent in the control population, it is at low frequency. We initially tested these plants after cultivation for 4 months in the greenhouse, and our results did not differ significantly from those obtained after 18 months of cultivation. Although progeny tests would be more conclusive, the fact that tolerance is not lost in cultivation suggests to us that it is a genetically controlled character and, together with the failure of control tillers to root in low concentrations of arsenic, seems to rule out habituation.

The degree of arsenic tolerance of each of the mine plants is related to the amount of arsenic in the soil. It is not surprising that plants from the most arsenical soils exhibit the greatest tolerance since selection would eliminate less tolerant individuals. However, it seems remarkable that few highly tolerant plants are found in the areas of low soil arsenic. Metal-tolerant plants of several species have been shown to be at a disadvantage on normal soil suggesting that nontolerant individuals are competitively superior on uncontaminated soils (*7*). We believe that competition on the areas of low soil arsenic may be great enough to exclude highly tolerant individuals. To test this hypothesis, we determined the number of plants of *A. scoparius* per square meter at each station. Our results, ranging from sparse (2 plants per square meter) to dense (11 plants per square meter), show that, except for station E-1, the lower the tolerance of each plant, the greater the number of plants per square meter. Thus, given the variation in density of plants in relation to the amount of soil arsenic, selection tends to favor highly tolerant individuals in extremely toxic areas and eliminate them in less toxic areas.

SUE ELLEN ROCOVICH
DAVID A. WEST

Department of Biology,
Virginia Polytechnic Institute and
State University, Blacksburg 24061

References and Notes

1. D. Jowett, *Evolution* **18**, 17 (1964); A. D. Bradshaw, T. S. McNeilly, R. P. G. Gregory, *Br. Ecol. Soc. Symp.* **5**, 327 (1965); T. S. McNeilly and A. D. Bradshaw, *Evolution* **22**, 108 (1968); T. S. McNeilly and J. Antonovics, *Heredity* **23**, 205 (1968).
2. R. P. G. Gregory and A. D. Bradshaw, *New Phytol.* **64**, 131 (1965).
3. H. F. Clements and J. Munson, *Pac. Sci.* **1**, 151 (1947).
4. D. A. Wilkins, *Nature (Lond.)* **180**, 37 (1957).
6. J. Antonovics, A. D. Bradshaw, R. G. Turner, *Adv. Ecol. Res.* **7**, 1 (1971).
7. T. S. McNeilly, *Heredity* **23**, 99 (1968); S. C. A. Cook, C. Lefébvre, T. S. McNeilly, *Evolution* **26**, 366 (1972).
8. We thank Dr. J. R. Craig for assistance in soil analysis, and Drs. J. R. Craig, E. R. Stout, and J. G. Waines for critical comments. Supported by a Small Projects grant from the College of Arts and Sciences and by the Department of Biology, VPI and SU.

13 November 1974; revised 10 January 1975

Insulin Receptor: Role in the Resistance of Human Obesity to Insulin

Abstract. *Large adipocytes from obese subjects have similar receptor numbers and affinities for insulin as small adipocytes from subjects of normal weight. These results indicate that the insulin insensitivity of large fat cells from obese humans occurs after the insulin-receptor interaction and might be explained by either a dilution of receptors over the cell surface or by alterations in intracellular metabolism.*

Hyperinsulinemia in the presence or absence of glucose intolerance is a universal accompaniment of obesity and suggests peripheral resistance to the action of insulin. In obese subjects direct correlations have been made between the degree of adiposity (*1*), the increased size of fat cells (*2*), and the elevated plasma insulin. Impaired action of insulin has been demonstrated in human subcutaneous adipose tissue from obese subjects in vivo in studies of perfused forearms (*3*) and in some (*4–6*) but not all (*7*) in vitro studies of adipose tissue and isolated adipocytes. Following the decrease in plasma insulin and the size of fat cells that accompany reduction in weight, the responsiveness of adipose tissue to insulin is restored (*4*).

Insulin initiates its action on target tissues by interacting with specific receptors on the cell membrane (*8, 9*). The insensitivity of large fat cells to insulin could result from a decrease in the number of insulin binding sites on the cell membrane, a lower affinity of binding, or a defect following the insulin-cell interaction. In a recent study Olefsky *et al.* (*10*) measured insulin binding to adipocytes from subjects of normal weight. The present report describes the first detailed study that compares the number and affinity of insulin receptors in adipocytes from both normal and obese human subjects.

Fat tissue for these studies was obtained from markedly obese subjects and from those of normal weight (Table 1). All obese subjects (five fe-

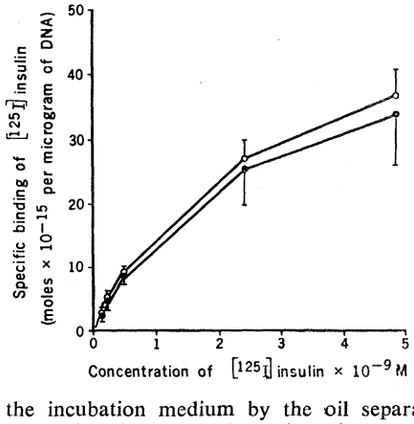

Fig. 1. Specific binding of [^{125}I]insulin to large and small human adipocytes. Following the surgical removal of subcutaneous adipose tissue, large fat cells (●) from obese subjects and small fat cells (○) from normal weight subjects were prepared by gently shaking at 37°C for 45 minutes in Krebs-Ringer bicarbonate buffer (*p*H 7.4) containing crude collagenase (1 mg/ml) and albumin (4 percent). Large (0.3 to 1.4 μg of DNA) and small (0.6 to 2.7 μg of DNA) cells were incubated for 40 minutes at 24°C in 0.3 ml of Krebs-Ringer bicarbonate buffer containing 1 percent albumin and the designated concentrations of [^{125}I]insulin. The fat cells were separated from the incubation medium by the oil separation method of Gliemann *et al.* (*26*), and the radioactivity was determined in a gamma counter. DNA was determined by the diphenylamine reaction (*27*). The [^{125}I]insulin was prepared by iodination with "carrier-free" Na^{125}I with the use of chloramine-T as described by Cuatrecasas (*9*). Specific binding is calculated as the difference between the total binding of [^{125}I]insulin in the absence of native insulin and in the presence of a large excess of unlabeled insulin (50 μg/ml)