Sandhoff-Jatzkewitz disease (*6*), generalized gangliosidosis (*24*), Fabry's disease (*25*), and metachromatic leukodystrophy (*4*).

The significance of our experiments is (i) the demonstration of an inherited single mutation of murine β-glucuronidase which alters the electrophoretic mobility of the enzyme localized at two different subcellular sites and (ii) the support that this variant lends to a model in which the multiple molecular forms of an acid hydrolase are coded by a single structural gene whose gene product undergoes secondary modifications.

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References and Notes

1. R. O. Brady, W. G. Johnson, B. W. Uhlendorf, *Am. J. Med.* **51**, 423 (1971).
2. R. Ganschow and K. Paigen, *Genetics* **59**, 335 (1968); D. Robinson, R. G. Price, N. Dance, *Biochem. J.* **102**, 525 (1967); E. Beutler and W. Kuhl, *Am. J. Hum. Genet.* **24**, 237 (1972); T. B. Shows and P. A. Lalley, *Biochem. Genet.* **11**, 121 (1974).
3. D. Robinson and J. L. Stirling, *Biochem. J.* **107**, 251 (1968).
4. M. C. Rattazzi, J. S. Marks, R. G. Davidson, *Am. J. Hum. Genet.* **25**, 310 (1973).
5. A. Goldstone, P. Konecny, H. Koenig, *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **13**, 68 (1971).
6. K. Sandhoff, K. Harzar, W. Wässle, H. Jatzkewitz, *J. Neurochem.* **18**, 2469 (1971).
7. M. Carrol and D. Robinson, *Biochem. J.* **131**, 91 (1973).
8. K. Paigen, *Exp. Cell Res.* **25**, 286 (1961).
9. R. Ganschow and B. Bunker, *Biochem. Genet.* **4**, 127 (1970).
10. K. Paigen and W. K. Noell, *Nature (Lond.)* **190**, 148 (1961); R. L. Sidman and M. C. Green, *J. Hered.* **56**, 23 (1965).
11. R. Dofuku, U. Tettenborn, S. Ohno, *Nat. New Biol.* **234**, 259 (1971).
12. R. Swank and K. Paigen, *J. Mol. Biol.* **77**, 371 (1973).
13. R. Ganschow, in *Metabolic Conjugation and Metabolic Hydrolysis*, W. H. Fishman, Ed. (Academic Press, New York, in press).
14. C. R. Shaw, *Brookhaven Symp. Biol.* **17**, 117 (1964).
15. J. J. Hutton, *Biochem. Genet.* **3**, 507 (1969).
16. V. M. Chapman, F. H. Ruddle, T. H. Roderick, *ibid.* **5**, 101 (1971).
17. T. H. Roderick, F. H. Ruddle, V. M. Chapman, T. B. Shows, *ibid.*, p. 457.
18. R. J. DeLorenzo and F. H. Ruddle, *ibid.* **4**, 259 (1969).
19. J. J. Hutton and T. H. Roderick, *ibid.*, p. 339.
20. R. Ganschow and K. Paigen, *Proc. Natl. Acad. Sci. U.S.A.* **58**, 938 (1967).
21. T. Karl and V. Chapman, *Biochem. Genet.*, in press.
22. S. Okada and J. S. O'Brien, *Science* **165**, 698 (1969).
23. P. A. Lalley, M. C. Rattazzi, T. B. Shows, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 1569 (1974).
24. S. Okada and J. S. O'Brien, *Science* **160**, 1002 (1968).
25. J. A. Kint, *ibid.* **167**, 1268 (1970).
26. T. B. Shows, F. H. Ruddle, T. H. Roderick, *Biochem. Genet.* **3**, 25 (1969).
27. M. Hayashi, Y. Nakajima, W. H. Fishman, *J. Histochem. Cytochem.* **12**, 293 (1964).
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Detection of a Gonadotropin in Rabbit Blastocyst before Implantation

Abstract. *A gonadotropin similar to human chorionic gonadotropin or luteinizing hormone has been demonstrated in rabbit blastocyst prior to implantation. The gonadotropin has been detected by a radioreceptor assay for human chorionic gonadotropin with the use of the plasma membranes of bovine corpora lutea obtained during the first trimester of pregnancy. The concentrations of the human chorionic gonadotropin or luteinizing hormone per milliliter of blastocyst fluid were tenfold higher than those in the blood of pregnant rabbits on days 5 and 6 after mating.*

The detection of pregnancy as early as days 6 to 8 after conception (*1*) suggested to us that we investigate the possible presence of a substance similar to human chorionic gonadotropin (hCG) or luteinizing hormone (LH) in the blastocyst prior to implantation. The existence of pregnancy was determined by the measurement of hCG-like or LH-like material in the blood by a radioreceptor assay for hCG, on the basis of competitive protein binding (*2, 3*).

Ten albino virgin rabbits were mated. On day 6 after mating, the animals were killed. The uterine horns were dissected on a petri dish and flushed with physiological saline by the aid of a syringe. The blastocysts, approximately three to ten from each rabbit, were transferred to a test tube with a Pasteur pipet. The fluid adhering to the blastocysts was drawn off. The blastocysts were disrupted by a needle and centrifuged. The volume of the supernatant was approximately 15 μl per blastocyst. The supernatant was tested in the radioreceptor assay of hCG-LH concentrations of 10, 20, 50, and 100 μl. Samples of plasma from nonpregnant and pregnant rabbits were also tested in the radioreceptor assay as follows. Samples of 100 μl of standard solution of hCG (12,000 international units per milligram) in doubling dilution [for example, 3.0, 6.3, 12.5, 25, 50, and 100 ng per milliliter of 10 m*M* tris-HCl buffer (*p*H 7.2) containing 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 0.1 percent bovine serum albumin], 10 international units of Trasylol in 20 μl of tris-HCl buffer, plasma membranes (*3*) (25 mg of protein in 100 μl incubation buffer), and ^{125}I (approximately 50,000 count/min in 100 μl of incubation buffer; specific activity, 40 to 50 μc/μg) were added to disposable plastic vials (75 × 100 mm) to both standard and unknown samples. The reaction mixture was then incubated at 37°C for 20 minutes. The tubes were placed in an ice bath, and 1 ml of chilled tris-HCl buffer was added to each tube. The contents of the tube were mixed on a Vortex mixer and centrifuged for 10 minutes at 5000*g*. The supernatants were aspirated, and the radioactivity bound to the plasma membranes was counted in an autogamma counter (Packard Instruments) with a 51 percent efficiency for ^{125}I.

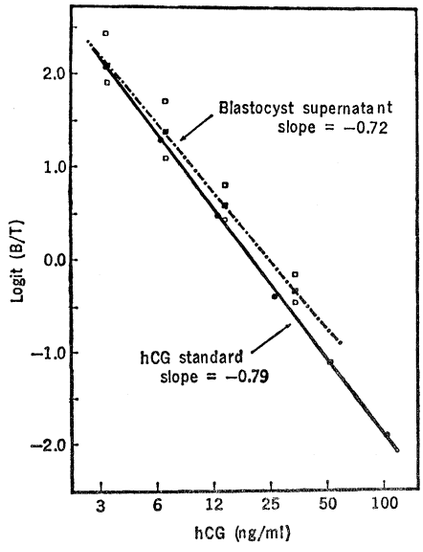

Fig. 1. Dose-response curve for human chorionic gonadotropin (hCG) and the supernatant of the ruptured and centrifuged blastocysts expressed as logit-log transformation. The ratio B/T is the ratio of ^{125}I-labeled hCG bound in the membrane receptor to the total ^{125}I-labeled hCG. Open squares represent duplicate experiments.

The dose-response curve for hCG and blastocyst supernatant are presented as logit-log transformations (*2*) in Fig. 1. Increasing quantities of the blastocyst supernatant progressively inhibited the binding of ^{125}I-labeled hCG, yielding a slope of −0.72, a value which is close to that of the hCG standard of −0.79, suggesting the presence of a material resembling hCG or LH in the blastocyst fluid. Ten assays performed on 50 blastocysts yielded, per

milliliter of supernatant, an average of 87 ± 17 ng of material resembling hCG or LH or approximately 1 to 1.5 ng per blastocyst. These estimates are tentative, but they provide unequivocal evidence for the presence of material similar to hCG or LH in the rabbit blastocyst prior to implantation.

In the plasma of pregnant rabbits on days 5 and 6 after mating the concentration of material resembling hCG or LH was 6 to 8 ng/ml, values significantly higher than the < 3 ng/ml found in the plasma of nonpregnant animals. Since the radioreceptor assay does not discriminate between hCG and LH, the release of pituitary LH after fertilization was also considered. However, the pituitary as a single source of LH would probably not explain the tenfold difference in concentrations of the gonadotropins between blastocyst and serum. These observations are unique in view of the existing concept that hCG is produced by syncytiotrophoblast after the implantation of the blastocyst. There is some evidence for the secretion of LH-like material by the rabbit blastocyst as measured by radioimmunoassay (*4*), as well as for high concentrations of hCG around the time of implantation as measured by a specific radioimmunoassay of the hormone-specific β subunit (*5*). In our study, the radioreceptor assay recognized only native hormone and thus interference by nonspecific immunoreactive material was eliminated. Hence the gonadotropin in the blastocyst may be similar to both hCG and LH. It is known that rabbit blastocyst is capable of synthesizing certain steroids and enzymes (*6*). Since trophoblast is differentiated as early as at the 17-cell stage (*6*), it is conceivable that these cells may be capable of producing a gonadotropin at very early stages of the development of the rabbit blastocyst. An hCG- or LH-like material has also been detected in the uterine fluid. Hence, the actual site of synthesis of gonadotropins before implantation remains to be established. However, this finding suggests the role of preimplantation gonadotropin in the maintenance of corpus luteum function in early pregnancy (*7*). The hCG has been shown to be a potent and reversible inhibitor of the response of human lymphocytes to phytohemagglutinin (*8*). Also activity similar to that of blood group A in the α subunit of hCG, before and after the removal of carbohydrate moiety, has been reported (*8*). These findings may have bearing on the possible role of hCG-like gonadotropin in the suppression of lymphocyte function during implantation.

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References and Notes

1. B. B. Saxena and K. Schmidt-Gollwitzer, in *Proceedings of the International Round Table Conference on Hormones in Pregnancy, Fresnes, France, October, 1973*, R. Scholler, Ed. (SEPE, Paris, 1974); B. B. Saxena, S. H. Hasan, F. Haour, K. Schmidt-Gollwitzer, *Science* **184**, 793 (1974).
2. S. A. Berson and R. S. Yalow, *J. Clin. Invest.* **38**, 1996 (1959); D. Rodbard, in *Receptor for Reproductive Hormones*, B. W. O'Malley and A. R. Means, Eds. (Plenum, New York, 1973), p. 289.
3. F. Haour and B. B. Saxena, in *Proteides of the Biological Fluids*, H. Peters, Ed. (Pergamon, New York, 1973); *J. Biol. Chem.* **249**, 2195 (1974).
4. S. Fugimoto, H. D. Woody, W. R. Dukelow, *Fed. Proc.* **32**, 214 (1973).
5. G. D. Braunstein, J. M. Grodin, J. Vaitukaitis, G. T. Ross, *Am. J. Obstet. Gynecol.* **115**, 447 (1973).
6. J. Davies, H. Hesseldahl, E. S. E. Hafez, N. O. Owers, R. J. Blandau, in *The Biology of the Blastocyst*, R. J. Blandau, Ed. (Univ. of Chicago Press, Chicago, 1971), pp. 27, 139.
7. F. Haour, B. B. Saxena, G. W. Cooper, S. Fugimoto, A. R. Fuchs, 56th Endocrine Society Meeting, Atlanta, 1974, abstr. 223; A. R. Fuchs, *ibid.*, abstr. 224; C. Beling, A. R. Fuchs, F. Haour, K. Park, B. B. Saxena, abstracts of Steroid Congress, Mexico, 1974.
8. E. W. Adcock, F. Teasdale, C. S. August, S. Cox, G. Meschia, F. C. Battaglia, M. A. Naughton, *Science* **181**, 845 (1973); L. Marz, O. P. Bahl, J. F. Mohn, *Biophys. Biochem. Res. Commun.* **55**, 717 (1973).
9. We thank Dr. G. Cooper, Dr. S. Fugimoto, and A. R. Fuchs for samples and N. Moore for technical assistance. Supported by NIH contract NICH D-72-2763 and grants CA-13908 and HD-06543; and grant 670-0455A from Ford Foundation. B.B.S. is a career scientist awardee of the Health Research Council, City of New York, contract 1-621. F.H. is partially supported by a fellowship from the Population Council (Rockefeller University, New York).

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Dedifferentiated Guard Cells in Magnoliaceous Leaves

Abstract. *Evidence has been obtained that guard cells and other epidermal cells as well as mesophyll cells undergo division during wound repair of mature leaves in 26 magnoliaceous taxa in the genera* Kmeria, Elmerrillia, Magnolia, Manglietia, Michelia, Paramichelia, *and* Talauma. *Division of epidermal cells is believed to be rare in mature leaves, and division of guard cells is particularly unusual in most species previously studied.*

Epidermal cells do not usually divide during regeneration after wounding in mature leaves. Internal cells of the leaf blade commonly exhibit an immediate response to injury. Cells along the wound die, and adjacent living cells are induced to undergo rapid physiological changes (*1*). Subsequent stages of wound repair may include cell proliferation, callus development, periderm formation, and cork differentiation. Most commonly, epidermal participation is nil (*2*); other responses reported include division only in immature epidermal cells (*3*), or division in epidermal components under pathological conditions only, as in gall formation (*4, 5*). Dehnel (*6*) and Wylie (*2*) are the only workers who have reported cell division in epidermis in wound repair zones of otherwise healthy, mature leaves. Von Mohl (*7*) cited periderm formation in *Buxus* leaf epidermis, which implies cell division. Reports of cell division in guard cells are even more rare (*4, 8*).

I have studied leaf regeneration following experimental wounding in two magnoliaceous species and following natural wounding in herbarium specimens of about 52 magnoliaceous species, representing 10 of the 11 genera in the family. Leaves were prepared for study by bleaching in 5 percent sodium hydroxide, clearing in saturated aqueous chloral hydrate, and staining in safranin (*9*), which makes it possible to observe the wound area at all levels of the leaf thickness and to compare it with healthy tissue in other parts of the same leaf. Results indicate that the mature epidermis dedifferentiated (underwent cell division and returned to a less differentiated state) during wound repair in leaves of 65 percent of the species studied; in the remaining 35 percent only internal leaf tissues participated in regeneration. In all the taxa studied, the mesophyll was the primary site of regeneration, rather than the epidermis or vascular tissue. The mesophyll cells adjacent to the necrotic zone along the wound divided repeatedly and in various planes to produce a callus of uniformly small, isodiametric cells. Intercellular spaces were greatly reduced compared to those in normal leaf tissue. In some instances the mesophyll cells eventually began to divide primarily periclinally to the wound surface.

There is indirect evidence that cell