the lower limit from Golgi observations being only 20 μm for β cells and 180 μm for α cells (*1*). The field diameters of some of the reconstructed dendrites were not so far from this: dendritic field diameters of two reconstructed β cells reached 25 and 28 μm, and that of a reconstructed α cell reached 50 μm. Most encouraging, the reconstructions provided more than enough detail for recognizing, at the electron microscopic level, soma sizes and the patterns and laminar distributions of dendrites described with light microscopy. The fact that each of the reconstructed neurons could be placed in one of the five major categories of ganglion cells means that the microdistribution of ganglion cells can now be studied in a systematic manner.

The greatest reward of the reconstructions was that they revealed the distribution of synaptic contacts on each cell. It was surprising to find, for example, that the α and β cells had few contacts on their somas and that the contacts on the dendrites of a particular cell tended to be restricted to the IPL sublamina in which its major arborization was contained. The distribution of synaptic contacts seems to be specific for each class, supporting the overall classification scheme. These findings also support and extend similar work by Kolb (*9*).

The next tasks are to identify the sources of the synaptic contacts by tracing them through a series of sections back to their cells of origin, to identify the transmitter chemistry of these presynaptic elements, and to analyze the cable properties of the ganglion cells and their inputs in order to understand the effects of the different soma surface areas and dendritic geometries. Contributions toward these ends made with the reconstruction approach are presented elsewhere (*10*).

JOHN K. STEVENS*
Department of Physiology, University of Pennsylvania School of Medicine, Philadelphia 19104
BARBARA A. MCGUIRE
PETER STERLING
Department of Anatomy, University of Pennsylvania School of Medicine

References and Notes

1. B. B. Boycott and H. Wässle, *J. Physiol. (London)* **240**, 397 (1974). See also A. Hughes, J. Stone, Y. Fukuda [*J. Neurophysiol.* **37**, 722 (1974)], and A. Hughes [*J. Comp. Neurol.* **163**, 107 (1975)].
2. C. Enroth-Cugell and J. Robson, *J. Physiol. (London)* **187**, 517 (1966); B. G. Cleland, M. W. Dubin, W. R. Levick, *ibid.* **217**, 473 (1971); Y. Fukuda, *Vision Res.* **11**, 209 (1971); J. Stone and K.-P. Hoffman, *Brain Res.* **43**, 610 (1972); J. K. Stevens and G. L. Gerstein, *J. Neurophysiol.* **39**, 213 (1976).
3. R. Nelson, E. V. Famiglietti, H. Kolb, *J. Neurophysiol.* **41**, 472 (1978).
4. W. Rall, *ibid.* **30**, 1138 (1967); O. F. Schanne and E. P. Ceretti, *Impedance Measurements in Biological Cells* (Wiley, New York, 1978); J. N. Barrett and W. E. Crill, *J. Physiol. (London)* **239**, 301 (1974); *ibid.*, p. 325; H. D. Lux, P. Schubert, G. W. Kreutzberg, in *Excitory Synaptic Mechanisms*, P. Anderson and J. K. S. Jansen, Eds. (Universitetsforlaget, Olso, 1970), p. 189.
5. The retina was taken from an adult cat perfused with buffered aldehydes and was prepared for electron microscopy in the standard manner. Silver-gold sections (100 nm) were stained with uranium and lead. We determined that the block was within 1° to 2° of the area centralis by comparing the density of ganglion cells prepared from a series of 2-μm sections that passed completely through the area centralis.
6. Two overlapping electron micrographs were made of each section at ×1300, the lowest magnification adequate for resolving synaptic contacts. A cell was reconstructed by tracing its outline from successive prints (final magnification, ×3400) onto separate acetate sheets and, using appropriate fiducial marks, aligning the sheets on a cartoonist's jig. Synaptic contacts were identified by viewing the prints through a dissecting microscope at a maximum magnification of ×120,000. Contacts were defined as sites of vesicle accumulation associated with a presynaptic ribbon or with increased density of presynaptic and postsynaptic membranes.
7. We chose the profile in which the cell's cross-sectional area was greatest and divided it by a series of 30 to 50 chords that were parallel to the retinal surface and orthogonal to the plane of section; the longest chord approximated the flat-mount diameter. An equivalent cylinder was generated for each chord; the sum of their surface areas and the sum of their volumes provided estimates of the soma surface area and volume, respectively. Although the values are given in micrometers (Table 1), shrinkage makes them less than the actual values.
8. S. Ramon y Cajal, *The Structure of the Retina*, compiled and translated by S. A. Thorpe and M. Glickstein (Thomas, Springfield, Ill., 1972).
9. H. Kolb, *J. Neurocytol.* **8**, 295 (1979).
10. S. Ellias and J. K. Stevens, in preparation; Y. Nakamura, B. A. McGuire, P. Sterling, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
11. Supported by National Eye Institute grants EY00828, EY01832, and EY01583. We thank D. Netsky and T. Davis for their help, B. Woolsey and J. Woolsey for their artistic rendering of the reconstructions, and H. Abriss for typing the manuscript. We are particularly indebted to F. Letterio and E. Shalna for their technical support.
* Present address: PlayFair Neuroscience Unit, Toronto Western Hospital, Toronto, Ontario, Canada M5T 2S8.

19 September 1979; revised 25 October 1979

Reduction of the Isoproterenol-Induced Alterations in Cardiac Adenine Nucleotides and Morphology by Ribose

Abstract. *Continuous intravenous infusion of ribose (200 milligrams per kilogram per hour) for 24 hours induced a marked stimulation of cardiac adenine nucleotide biosynthesis in unanesthetized and unrestrained rats that had been treated with isoproterenol subcutaneously (25 milligrams per kilogram). The diminution of adenine nucleotides characteristic for the action of high doses of isoproterenol was entirely prevented, and the incidence of the isoproterenol-induced myocardial cell damage was significantly reduced when ribose was administered. These results support the view that depletion of adenine nucleotides is involved in the development of cardiac necrosis.*

Isoproterenol, a synthetic catecholamine, is well known for its pronounced positive inotropic effect mediated by an increased transmembrane Ca^{2+}-influx into the myocardial cell (*1*, *2*). Because of an excessive consumption of adenosine triphosphate (ATP), the cardiac adenine nucleotide pool becomes diminished under these conditions (*1*, *3*). It is this ATP decrease that has been considered responsible for the development of myocardial cell lesions (*3*) occurring after application of high doses of isoproterenol in rats (*4*). Various attempts have been made to prevent or reduce the isoproterenol-induced cardiac cell damage, including administration of high potassium and low sodium diets (*5*), application of β-receptor-blocking agents (*6*), and treatment with calcium-antagonistic compounds and calcitonin, and acidification (*3*). These interventions tend to attenuate the positive inotropic effect of isoproterenol and to prevent the diminution of cardiac ATP, thus maintaining the structural integrity of the myocardium.

Table 1. Concentrations of ATP and of adenine nucleotides (AN; sum of ATP, ADP, AMP) and rates of AN biosynthesis in the myocardium of rats under control conditions and 24 hours after subcutaneous application of isoproterenol (25 mg/kg), with constant intravenous infusion of either 0.9 percent NaCl or ribose (200 mg/kg per hour) for 24 hours. Results are given as mean values ± the standard error of the mean; the numbers in parentheses indicate the number of experiments.

Isoproterenol addition	ATP (nmole/g)	AN (nmole/g)	AN change after isoproterenol (nmole/g per 24 hours)	Biosynthesis of AN (nmole/g per 24 hours)
None	4470 ± 84 (34)	5893 ± 103 (34)		6.0 ± 0.7 (25)
NaCl	3167 ± 62* (9)	4190 ± 84* (9)	−1703	22.5 ± 3.7* (4)
Ribose	4565 ± 292 (6)	6047 ± 208 (6)		80.1 ± 11.1* (8)

*$P < .005$ compared with the control values

0036-8075/80/0118-0319$00.50/0

In an attempt to antagonize the deleterious effects of isoproterenol on adenine nucleotide content and structure of the hearts, we have designed a different experimental approach to counterbalance the decrease of the ATP concentration by stimulating the biosynthesis (de novo synthesis) of cardiac adenine nucleotides. This approach is based on the facts that adenine nucleotide biosynthesis in the rat heart in vivo is enhanced by isoproterenol (*7*), that it is limited by the flow through the hexose monophosphate shunt, and that this limitation can be overcome by ribose or other pentoses and pentitols (*8*). Ribose, which bypasses the hexose monophosphate shunt, is immediately converted to ribose 5-phosphate, thus giving rise to an elevation of the available pool of 5-phosphoribosyl-1-pyrophosphate, an essential precursor substrate for the biosynthesis of adenine nucleotides. As a result, adenine nucleotide synthesis is enhanced under these conditions (*8*). We have administered a constant intravenous infusion of ribose for 24 hours to unanesthetized and unrestrained rats (*9*) that were first treated with isoproterenol, and have examined the effect of ribose infusion on adenine nucleotide metabolism and morphology of the hearts. We now report that cardiac adenine nucleotide biosynthesis in isoproterenol-treated rats is stimulated by ribose to such an extent that the diminution of adenine nucleotide levels induced by isoproterenol is prevented and the incidence of myocardial cell damage is reduced.

All experiments were done on female Sprague-Dawley rats (200 to 220 g) that were injected subcutaneously with isoproterenol (25 mg/kg) and then subjected to a constant intravenous infusion of either 0.9 percent NaCl or ribose (200 mg/kg per hour). At the end of a 24-hour infusion period (5 ml/kg per hour), the rats were anesthetized with diethyl ether and thoracotomized. The hearts were immediately excised and immersed in (i) Freon at −156°C when the concentration of adenine nucleotides was to be determined (*10*) or in (ii) 7 percent formaldehyde for histologic examination. For the latter, the hearts were assigned random code numbers, and slices (8 to 10 μm thick) obtained from at least eight differently localized cross sections were stained with hematoxylin-eosin and examined for myocardial cell lesions. In experiments designed to measure the biosynthesis of adenine nucleotides, the rats were intravenously injected with [1-^{14}C]glycine (0.25 mCi/kg in 1 ml of 0.9 percent NaCl) 1 hour before the end of the 24-hour infusion period. At the end of the infusion period, the rats were anesthetized with diethyl ether, and the hearts were promptly excised and frozen in liquid nitrogen. Rates of adenine nucleotide biosynthesis were calculated from the total radioactivity of cardiac adenine nucleotides and the mean specific activity of the intracellular glycine (*11*).

Table 2. Incidence of myocardial cell lesions after subcutaneous administration of isoproterenol (25 mg/kg) in rats with constant intravenous infusion of either 0.9 percent NaCl or ribose (200 mg/kg per hour) for 24 hours.

Isoproterenol addition	Animals (No.)	Myocardial damage (No.)
NaCl	17	16
Ribose	22	6

Isoproterenol induced a significant decline in the concentration of myocardial ATP as well as of the total adenine nucleotide pool in rats that had received constant intravenous infusion of 0.9 percent NaCl. When ribose was administered for 24 hours in isoproterenol-treated animals, the concentration of cardiac adenine nucleotides was normal (Table 1). The biosynthesis of adenine nucleotides was enhanced in hearts of isoproterenol-treated rats and turned out to be much higher when ribose was infused. If we assume that adenine nucleotides are synthetized at the same rate during the entire period of 24 hours as measured during the last 60 minutes of the 24-hour infusion period, we can calculate that the adenine nucleotides synthesized de novo in rats treated only with isoproterenol (540 nmole/g for 24 hours) are insufficient to compensate for the loss of about 1700 nmole/g brought about by isoproterenol. However, in hearts of isoproterenol-treated rats with constant intravenous infusion of ribose, about 1900 nmole of adenine nucleotides per gram would have been formed as a result of de novo synthesis within 24 hours. Such an amount can be expected to counterbalance the isoproterenol-induced diminution of cardiac adenine nucleotides. Thus it appears that the biosynthesis of myocardial adenine nucleotides can be sufficiently stimulated by ribose so that the heart is protected from energy deficiency that regularly occurs as a result of treatment with isoproterenol. A similar restitution of adenine nucleotides has been reported to be achieved by adenosine infusion in rabbit myocardium during postasphyctic recovery (*12*). The mechanism involved in the stimulation of adenine nucleotide biosynthesis in our experimental conditions appears to be a combined effect of an increased availability of 5-phosphoribosyl-1-pyrophosphate brought about by ribose and of release of feedback inhibition of 5-phosphoribosyl-1-pyrophosphate amidotransferase (E.C. 2.4.2.14), the first and rate-limiting enzyme of the de novo synthesis, induced by isoproterenol.

The ATP decrease is a good indicator of the degree of injury of the heart, and it plays a decisive role in the development of myocardial cell damage (*1, 3, 13*). Thus, if the depletion of ATP is prevented by ribose, as we have shown, then the isoproterenol-induced cardiac cell lesions should be reduced. In fact, 73 percent of the isoproterenol-treated rats did not exhibit any sign of cardiac cell damage when ribose was constantly administered, whereas almost all rats injected with isoproterenol and maintained on intravenous infusion with 0.9 percent NaCl developed cardiac necrosis with mesenchymal infiltration (Table 2). This result lends further support to the hypothesis that the decrease of cardiac adenine nucleotides is an important factor involved in the development of the myocytolytic processes initiated by isoproterenol. On the other hand, ribose and other pentoses and pentitols may be considered to be of potential therapeutic advantage provided that they affect adenine nucleotide metabolism in the human myocardium in the same manner as they do in the rat heart.

H.-G. ZIMMER
H. IBEL
G. STEINKOPFF
Department of Physiology, University of Munich, 8000 Munich 2, Germany
G. KORB
Institute of Pathology, General Hospital, 8480 Weiden, Germany

References and Notes

1. A. Fleckenstein, in *Calcium and the Heart*, P. Harris and L. Opie, Eds. (Academic Press, London, 1971), pp. 135–188.
2. H. Reuter and G. W. Beeler, Jr., *Science* **163**, 399 (1969).
3. A. Fleckenstein, J. Janke, H. J. Döring, in *Recent Advances in Studies on Cardiac Structure and Metabolism*, N. S. Dhalla, Ed. (University Park Press, Baltimore, 1974), pp. 563–580.
4. G. Rona, C. I. Chappel, T. Balazs, R. Gaudry, *AMA Arch. Pathol.* **67**, 443 (1959); C. I. Chappel, G. Rona, T. Balazs, R. Gaudry, *Can. J. Biochem. Physiol.* **37**, 35 (1959); G. Korb and V. Totovic, *Virchows Arch. Pathol. Anat. Physiol.* **336**, 475 (1963); G. Korb, *ibid.* **339**, 136 (1965).
5. G. Rona, C. I. Chappel, D. S. Kahn, *Am. Heart J.* **66**, 389 (1963).
6. S. Bloom and D. Davis, in *Recent Advances in Studies on Cardiac Structure and Metabolism*, N. S. Dhalla, Ed. (University Park Press, Baltimore, 1974), pp. 581–590.
7. H.-G. Zimmer and E. Gerlach, *Circ. Res.* **35**, 536 (1974).

8. ——, *Pfluegers Arch.* **376**, 223 (1978).
9. H.-G. Zimmer and H. Ibel, *Experientia* **35**, 510 (1979).
10. E. Gerlach, B. Deuticke, R. H. Dreisbach, *Pfluegers Arch.* **278**, 296 (1963).
11. H.-G. Zimmer, C. Trendelenburg, H. Kammermeier, E. Gerlach, *Circ. Res.* **32**, 635 (1973).
12. W. Isselhard, D. H. Hinzen, E. Geppert, W. Mäurer, *Pfluegers Arch.* **320**, 195 (1970).
13. R. B. Jennings, H. K. Hawkins, J. E. Lowe, M. L. Hill, S. Klotman, K. A. Reimer, *Am. J. Pathol.* **92**, 187 (1978); J. Schaper, J. Mulch, B. Winkler, W. Schaper, *J. Mol. Cell. Cardiol.* **11**, 521 (1979).
14. Supported by a grant from the Deutsche Forschungsgemeinschaft (Zi 199/1).

25 July 1979; revised 15 October 1979

Origin of chi46,XX/46,XY Chimerism in a Human True Hermaphrodite

Abstract. *Using chromosome heteromorphisms and blood cell types as genetic markers, we demonstrated chimerism in a chi46,XX/46,XY true hermaphrodite. The pattern of inheritance of the chromosome heteromorphisms indicates that this individual was probably conceived by the fertilization, by two different spermatozoa, of an ovum and the second meiotic division polar body derived from the ovum and subsequent fusion of the two zygotes. This conclusion is based on the identification of the same maternal chromosomes 13, 16, and 21 in both the 46,XX and 46,XY cells of the patient. In the two cell lines of the chimera, chromosomal markers showed different paternal No. 9 chromosomes and sex chromosomes, as well as the same paternal chromosome 22.*

A chimera is an individual with two or more genetic cell types resulting from the fusion of different zygotes (*1*). Chimeras can be readily classified as whole-body or partial chimeras according to their mode of origin. Partial chimeras can arise by placental cross-fertilization between dizygotic twins, maternal-fetal transplacental exchange, transfusions, or grafting (*2, 3*). Because of lack of suitable studies, the origin of whole-body chimeras is less clear. Theoretically, they can arise by (i) early fusion of different embryos, (ii) fertilization of an ovum and any polar body by two different sperm and subsequent fusion of the zygotes, (iii) fertilization of a haploid ovum or polar body and subsequent fusion with a diploid polar body or ovum, or (iv) fusion of a diploid sperm with an embryo (*2*).

The origin of whole-body chimeras may be learned by comparing markers between the cell lines of the chimera and those of the parents and explaining their presence by oogenesis and spermatogenesis. Because most chimeras have been investigated with use of genetic markers that frequently cross over in meiosis, such as blood groups and cell types, and most cytogenetic studies have been done with nonbanding techniques, the specific origin for any whole-body chimera has not been established (*3, 4*). Almost all cases have had the karyotype chi46,XX/46,XY, but some diploid/triploid individuals may also be chimeras (*5, 6*). Thus, most reported chimeras have sexual abnormalities such as clitoral hypertrophy or true hermaphroditism. Chimeras of the type chiXX/XX or chiXY/XY probably exist but are clinically normal and only fortuitously discovered (*7*).

By using banding techniques cytogeneticists have demonstrated that there is considerable interindividual heteromorphism of satellites and certain bands among human chromosomes (*8*). These heteromorphisms are inherited in a Mendelian fashion, and meiotic crossing-over rarely occurs (*9*). Using chromosome heteromorphisms, we studied a true hermaphrodite who probably arose by fertilization of an ovum and its second meiotic division polar body.

Shortly after birth, this patient was referred for chromosome analysis because of ambiguous genitalia. Genital abnormalities included a prominent phallus, scrotalization of the labial-scrotal swelling, slight posterior fusion of the labia minora, and a single orifice at the base of the phallus. The plasma, electrolytes, and glucose were normal. The concentrations of 17-hydroxyprogesterone in the plasma and 17-ketosteroid and 17-ketogenic steroids in the urine were normal. Pregnanetriol was undetectable. At laparotomy, a rudimentary unicornate uterus, fallopian tubes, and a streaklike gonad containing oocytes were found on the left side and removed. On the right side, a fetal testis, vas deferens, and epididymis were found and removed. Thus, this individual showed characteristics of a true hermaphrodite. Clitoral reduction was performed.

For the cytogenetic studies we used GTG-, QFQ-, and C-banding techniques on cultures of peripheral blood lymphocytes and biopsy specimens of the skin, fetal testis, and ovarian gonad from the patient and on peripheral blood specimens of the parents(*10*). All tissues from the patient showed both 46,XX and 46,XY cells (Table 1). Heteromorphisms of chromosomes 9, 13, 16, 21, and 22, as well as the Y chromosome, proved to be informative (Fig. 1). One of the paternal No. 9 chromosomes stands out because its centromere is located so that about one-third of the C band is on the p arm and about two-thirds is on the q arm. One maternal chromosome 16 is different because it has a larger C band than its own homolog or either of the paternal No. 16 chromosomes.

The satellites on the paternal No. 13 chromosomes are similar to one another but distinct from those of the mother: their stalks are very short, with relatively dull fluorescent terminal chromatin, and there is a bright QFQ band at their bases. One of the maternal No. 13 chromosomes has satellites with relatively longer stalks and a larger terminal chromatin mass than the other. One maternal chromosome 21 is conspicuous because of rather large, bright fluorescent satellites. Its homolog has just a faint satellite. One of the paternal No. 21 chromosomes has relatively large satellites, but it does not fluoresce as brightly as the conspicuous maternal chromosome 21. The other paternal chromosome 21 has small satellites. One paternal chromosome 22 is distinguished by large, bright fluorescent satellites. Its homolog has small satellites, and the maternal No. 22 chromosomes have even less satellite material.

In the XY line of the chimera, the Y chromosome and chromosomes 13 and 21 are of paternal origin and the homologs are of maternal origin. In the XX line, the paternal markers 9, 13, and 21 can be identified, as can the maternal markers 13 and 21. The maternal marker 16 and paternal marker 22 are not evident in either of the cell lines of the patient.

We performed ABO, Rh, MNSs, Duffy, Kidd, and lymphocyte HLA typing concomitantly on cells from the patient and the parents with use of the same serum and incorporation of appropriate positive and negative controls. Of these, only the Kidd system proved informative. Five different serum samples were used for the Jk^a (Kidd antigen) typings, and results were confirmed twice. All red cell typings were examined microscopically for the presence of "free" or unagglutinated cells mixed with large agglutinates (so-called mixed-field typing). In the Jk^a typing of the patient's cells, virtually the same degree of mixed-field

0036-8075/80/0118-0321$00.50/0