Table 1. Trehalase activity in tissues of human males. Results are given as the maximum catalytic capacity in micromoles of glucose, produced per minute per gram of fresh tissue, at *p*H 6.2, and at 38°C.

Patient		Liver	Kidney
Age (yr)	Cause of death		
		Cortex	
62	Pulmonary carcinoma	0.04	0.7
48	Accident	0.08	1.2
68	Myocardial infarction	0.20	2.3
60	Myocardial infarction	0.37	2.2
		Whole	
43	Myocardial infarction	0.07	1.4
66	Thrombosis, with chronic renal disease and occlusion of renal arteries	0.015	0.22

load, or in diabetes, at least 5.5 μmole of trehalase activity would be required to guard against net accumulation of trehalose in the tubular cell or leakage of trehalose into the blood. Similar trehalase activities should be present in the kidneys of all animals for which such a mechanism of glucose reabsorption is postulated.

Sacktor's values (*2*) in human cortex (4.2, 2.4, and 0.75 μmole) and the values in Table 1 fall somewhat short of this requirement, but they may be sufficient to support the hypothesis because, of necessity, these kidneys were removed several hours after the patients died, which may have diminished enzyme activity. Interestingly, the kidney in which the renal artery was occluded had a very low activity.

Trehalase activity in liver was much lower than in kidney (Table 1), but higher than serum acitvity (0.00 to 0.01 μmole) in man (*1*).

A serious objection to the hypothesis is the absence of trehalase from the kidney of the cotton rat, the Wistar rat, the house cat, all birds and snakes, and most turtles and fishes (Table 2). The absence or near-absence of trehalase in the rat, the cat, and, in addition, in two goat species was also observed by Sacktor (*2*).

Kidneys of species which are capable of rapid trehalose synthesis, but which lack trehalase, might still be able to function according to the hypothesis, if trehalase were present in serum or in liver. Indeed, several mammals have an active trehalase in liver and serum (*1*), which could hydrolyze trehalose spilled into the serum by the kidney. However, the species that lacked trehalase in the kidney showed no activity in serum or in liver.

If rapid conversion of glucose to trehalose provides the steep concentration gradient which allows passive absorption of glucose, the ratio of trehalose to glucose in the tubular cell should be high. However, the kidneys of rabbits and rats did not contain measurable quantities of trehalose.

The role of trehalose as an intermediate in glucose transport was further investigated by isotope studies. New Zealand White rabbits (which show high renal trehalase activity) and Wistar rats (which have no renal trehalase activity) were anesthetized and injected in the heart with a 5 percent solution of uniformly labeled glucose-C^{14} (25 mg per kilogram of body weight); 10 and 20 minutes later, the kidneys were removed and immediately boiled to stop further enzyme action. The aqueous extracts were concentrated, deproteinized, de-ionized, and chromatographed on paper in a butanol, ethanol, water system (52:30:18). The area with the approximate R_F value of trehalose was eluted and contained 10 to 30 percent as much radioactivity as the glucose fraction. The suspected trehalose eluate was then mixed with carrier trehalose and boiled with HCl or incubated with fresh rabbit kidney extract until it was completely hydrolyzed to glucose. Upon rechromatography, this glucose fraction contained less than 1 percent of the radioactivity, the remaining 99 percent still being in the trehalose area of the paper. These experiments do not support the hypothesis (*2*) that trehalose is an intermediate in glucose transport through the kidney in species with or without renal trehalase activity.

Table 2. Trehalase activity in whole kidneys of mammals (other than man) and of some other vertebrates. Results are in micromoles of glucose produced per gram of fresh tissue at *p*H 6.2, and at 38°C.

Species	Samples (No.)	Whole kidney
Other mammals		
Armadillo (*Dasypus novemcinctus*)	2	20
Gray fox (*Urocyon cinereoargenteus*)	1	14
Cottontail rabbit (*Sylvilagus floridanus*)	1	12
Cotton mouse (*Peromyscus gossypinus*)	5	7–20
White laboratory mouse	5	4–8
Raccoon (*Procyon lotor*)	2	4–9
Eastern wood rat (*Neotoma floridana*)	2	0.1–0.5
Cotton rat (*Sigmodon hispidus*)	1	0.00
Wistar rat	4	0.00
House cat	2	0.00
*Other vertebrates**		
Birds (20 species)		0.00
Snakes (12 species)		0.00
Turtles (7 species)		0.0–1
Frogs and toads (3 species)		0.5–2.5
Fishes (6 species)		0.0–0.5

* The complete list is available from the author.

EMILE VAN HANDEL
Florida State Board of Health, Entomological Research Center, Vero Beach 32960

References and Notes

1. E. Van Handel, *Comp. Biochem. Physiol.* **26**, 561 (1968).
2. B. Sacktor, *Proc. Nat. Acad. Sci. U.S.* **60**, 1007 (1968); W. Grossman and B. Sacktor, *Science* **161**, 571 (1968).
3. J. E. Treherne, *J. Exp. Biol.* **35**, 297 (1958).
4. Supported by NIH grant AI-06587. The human tissues were provided by Dr. L. E. McHenry, and the animal tissues by Drs. H. Kale and J. Edman. The analyses were performed by J. E. Guira.

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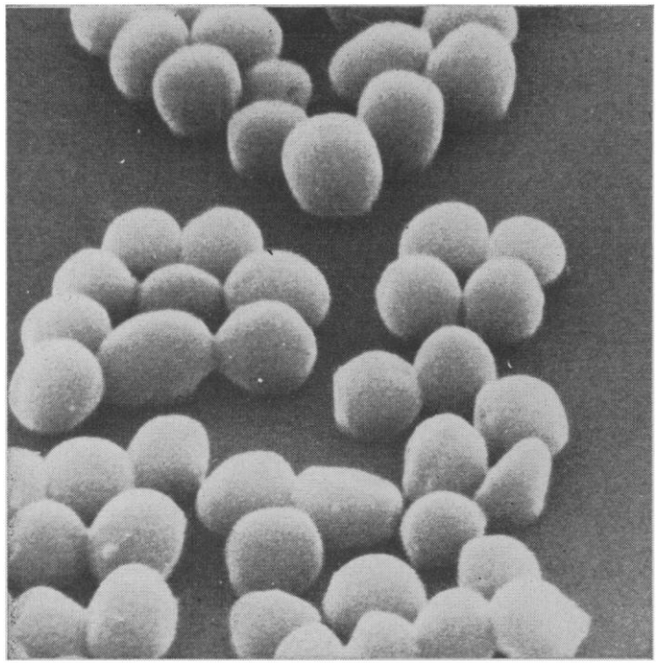
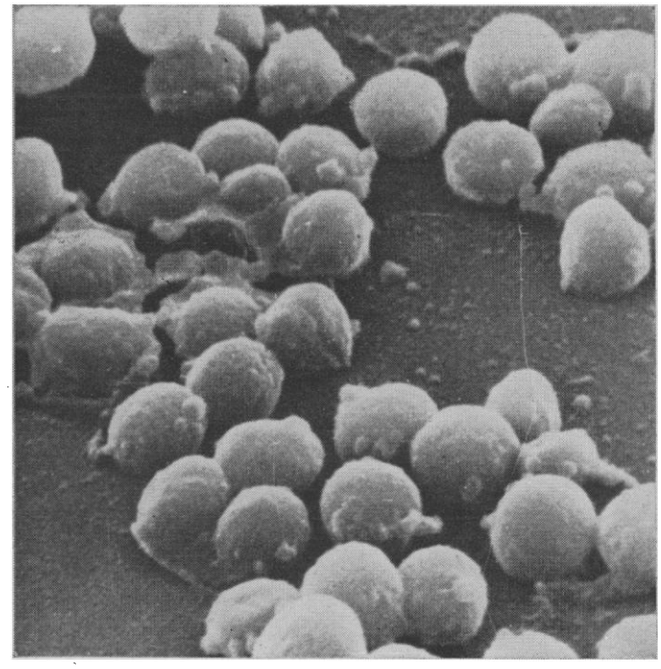
Antibiotic-Induced Surface Changes in Microorganisms Demonstrated by Scanning Electron Microscopy

Although available for several years, the scanning electron microscope has had little use by microbiologists. Bartlett (*1*) has examined surface structures of foraminifera, Williams and Davies (*2*) have studied actinomycetes, and Gray (*3*) has examined soil microorganisms.

Stereoscan electron microscopy permits the rapid examination of the surfaces of large numbers of organisms in three dimensions. Preparation of the material is relatively simple. The chief drawback to the machines now available is that the resolution (about 200 Å) is insufficient to resolve some of the surface structures, such as the flagella and fimbriae of some Gram-negative organisms. This considerably limits the value of the high magnifications obtainable, which are in excess of 100,000. Still, information about surface changes caused by antibiotics not obtainable with light microscopy or only laboriously reconstructed from electron microscopic studies can be obtained.

The Oxford strain of *Staphylococcus aureus* and a hemolytic streptococcus (Lancefield group A) obtained from clinical material, cultured in ordinary nutrient or glucose broth with or without ampicillin (100 μg/ml) for 1.5 to 2.5 hours, were fixed overnight in 1 percent glutaraldehyde in 5 percent sucrose solution. The suspensions were





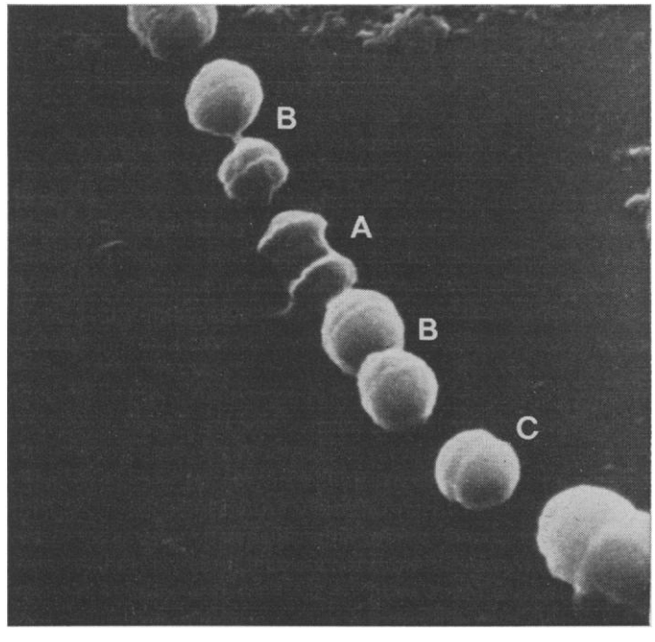


Fig. 1 (top left). *Staphylococcus aureus* (Oxford strain) before exposure to ampicillin (× 30,000).

Fig. 2 (top right). *Staphylococcus aureus* after exposure to 100 μg of ampicillin for 2.5 hours (× 30,000).

Fig. 3 (bottom left). *Streptococcus pyogenes* after exposure to 100 μg of ampicillin for 2 hours (× 35,000).

then washed three times (with light centrifugation) in distilled water, pipetted onto circular glass coverslips, and allowed to dry in air. The quality of pictures obtained was improved if the dried suspension was then treated with 5 percent ferric chloride, washed in several changes of distilled water, and air-dried once more. The specimens were coated and examined as described by Salsbury and Clarke (*4*) for red blood cells and by Williams and Davies (*2*) for actinomycetes.

The interference with cell-wall synthesis, which is the major effect of the penicillins, might uniformly affect the whole cell surface or produce discrete lesions—perhaps principally affecting incipient points of division. The *S. aureus* (Fig. 1) appeared to develop multiple points of cell weakness (Fig. 2), but these were not obviously concentrated along lines corresponding with cell septa.

The hemolytic streptococcus normally consists of a chain of diplococci (Fig. 3*C*). After exposure to ampicillin, walls of many cells collapsed giving an "apple core" appearance (Fig. 3*A*), while adjacent cocci in the chain were little affected (Fig. 3*B*). When the hemolytic streptococcus divides, the cocci first remain so that the chain consists of diplococci of which the adjacent zones are presumably undergoing maturation before final separation (*5*).

The appearance after exposure to ampicillin suggests that it is the adjacent separating areas of the cells which are principally affected (Fig. 3*A*), whereas the mature separated poles are relatively resistant—an appearance in keeping with the explanation postulated by Shrivastava and Thompson (*6*) for the hypersusceptibility of dividing organisms.

D. GREENWOOD
F. O'GRADY
Department of Bacteriology,
St. Bartholomew's Hospital,
London E.C.1, England

References and Notes

1. G. A. Bartlett, *Science* **158**, 1318 (1967).
2. S. T. Williams and F. L. Davies, *J. Gen. Microbiol.* **48**, 171 (1967).
3. T. R. G. Gray, *Science* **155**, 1668 (1967).
4. A. J. Salsbury and J. A. Clarke, *J. Clin. Pathol.* **20**, 603 (1967).
5. R. M. Cole, *Bacteriol. Rev.* **29**, 326 (1965).
6. R. B. Shrivastava and R. E. M. Thompson, *Brit. J. Exp. Pathol.* **48**, 315 (1966).

7. We thank Drs. A. J. Salsbury and J. A. Clarke, and the Cambridge Instrument Company for making available facilities for use of the Stereoscan electron microscope.

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Mammalian Oocytes: X Chromosome Activity

Abstract. *The glucose-6-phosphate dehydrogenase and lactate dehydrogenase contents of oocytes from XO and XX female mice have been measured. The activity of the former in the oocytes of XO mice is half of that in the oocytes of XX mice, whereas the lactate dehydrogenase activities in the two groups of ova are the same. These results indicate that glucose-6-phosphate dehydrogenase from a mouse oocyte is an X-linked enzyme, that its synthesis occurs in the oocyte and is dosage dependent, and that inactivation of the X chromosome does not occur in the mouse oocyte.*

Cytological and biochemical evidence indicates that only one X chromosome functions in any somatic cell of female mammals, so that males with only one X chromosome and females with two are functionally equivalent with regard to the gene products of the X chromosome (*1*). However, the two X chromosomes of mammalian oocytes do not behave in the same manner as those in the somatic cells. Rather than one of the X chromosomes appearing heteropycnotic (inactive) and the other isopycnotic (active), both are isopycnotic (*2*). If the cytological appearance of the chromosomes can be translated into physiological terms, both X chromosomes in the mammalian oocyte should be metabolically active. To test this hypothesis it is necessary to have a dosage-dependent X-linked marker and oocytes differing in the number of X chromosomes which they possess. These requirements are met by oocytes from the mouse *Mus musculus,* and our results indicate that both X chromosomes do function in mammalian oocytes.

Of the enzymes known to be present in the mouse ovum, the two present in the largest amount are lactate dehydrogenase (LDH) (E.C.1.1.1.27) and glucose-6-phosphate dehydrogenase (G6PD) (E.C.1.1.1.49) (*3*). The B subunits of LDH in man, deer mouse, and pigeon are under autosomal control, and it may be inferred that the B subunit present in the mouse oocyte is also under autosomal control (*4*); LDH thus is a useful index of autosomal activity.

Ohno has recently argued that all mammalian G6PD's are under control of the X chromosome since erythrocyte G6PD is X-linked in several quite remotely related mammalian orders, including man (*5*). Furthermore, G6PD from both deer mouse and human erythrocytes is functionally homologous, and that from rat and human erythrocytes is hybridizable in vitro, indicating structural homology (*6*). The X-linked G6PD must be distinguished from the autosomally controlled and less specific microsomal hexose phosphate dehydrogenase or glucose dehydrogenase (*7*). The G6PD in mouse oocytes has the same substrate specificity as erythrocyte G6PD, and both differ quite markedly from the hepatic microsomal hexose phosphate dehydrogenase (*8*).

Gene dosage holds for many autosomal loci of man and other mammals, and investigations of polyploid cells in mammalian liver have indicated that dosage probably holds for whole sets of chromosomes as well as for individual loci (*9*). If gene dosage applies to the synthesis of oocyte G6PD, and if oocyte X chromosomes are not inactivated, the content of G6PD should be proportional to the number of X chromosomes in the oocyte. This is what has been observed.

To obtain mouse oocytes differing in the number of X chromosomes they contain, XX and XO female mice were used (*10*). The XO and XX females are produced by crossing *Ta*/O females with B6CBAF_1(+) males. Tabby (*Ta*) is a marker used to follow segregation of the X chromosome. The female offspring of this cross are either *Ta*/+(XX) with a variegated phenotype or +/O (XO) with a wild-type phenotype. The XO females are also obtained by crossing *Ta*/Y males with +/O females to produce *Ta*/O females with the full tabby phenotype. Although the ovulated ova from XO females are either X or O in genotype after extrusion of the first polar body, oocyte LDH and G6PD are synthesized before this time, and all oocytes from XO females may be considered to have been XO in geno-

Table 1. Enzyme activities (mean ± S.E.) of mouse oocytes. The numbers of individual assays are noted in parentheses. Activities are expressed as nanomoles of substrate per hour per oocyte; *P* was calculated by Student's *t*-test.

Experiment	Genotype				Ratio of activities XO/XX	*P* for XO vs. XX
	XX	XO				
	Ta/+	*Ta*/O	+/O	Combined		
			Glucose-6-phosphate dehydrogenase			
1	1.64 ± .07 (17)	0.83 ± .05 (4)	0.83 (1)	0.83 ± .04 (5)	0.50	<.001
2	1.67 ± .10 (9)	.92 ± .03 (4)	.97 (1)	.93 ± .02 (5)	.56	<.001
3	1.54 ± .05 (10)	.80 (1)	.82 ± .06 (4)	.81 ± .04 (5)	.53	<.001
All experiments	1.62 ± .04 (36)	.86 ± .03 (9)	.84 ± .04 (6)	.86 ± .02 (15)	.53	<.001
			Lactate dehydrogenase			
2	34.5 ± 3.7 (7)	38.9 ± 1.4 (4)		38.9 ± 1.4 (4)	1.13	>.40
3	45.4 ± 2.1 (9)	45.5 ± 5.7 (2)	46.8 ± 2.7 (4)	46.4 ± 2.7 (4)	1.02	>.70
All experiments	40.7 ± 2.4 (16)	41.1 ± 2.2 (6)	46.8 ± 2.7 (4)	43.4 ± 1.9 (10)	1.06	>.40