maximal staining at $5 \times 10^{-3}M$ (Fig. 1). This finding is in good agreement with the K_m for fructose of rat hexokinases which are all similar in the range 3.1 to $4.0 \times 10^{-3}M$ (*12*).

The presence of glucokinase in human and dog liver and its responsiveness to the nutritional state emphasize the importance of this enzyme in the regulation of glucose utilization by the liver. The greater ease of demonstration of glucokinase in rat liver than in human liver may partly result from the eating habits of rats, which consume most of their food in the course of several hours during the night and thus present large amounts of substrate to the liver at one time. Moreover, the ordinary diet of rats contains only a small amount of fat and is chiefly carbohydrate, whereas the usual human diet contains considerable fat and less carbohydrate. Since glucokinase makes up most of the phosphorylating capacity of the liver in well-fed humans and dogs, as well as in rats, the level of this enzyme determines the capacity of the liver to dispose of glucose; thus the level determines the character of the glucose-tolerance curve. It is known that glucose tolerance declines with fasting, advancing age, and the presence of malignant disease. Our study has demonstrated that glucokinase is low in or absent from poorly nourished humans and dogs, and we anticipate that similar correlation may be possible with advancing age or malignancy in man.

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References and Notes

1. D. L. Di Pietro and S. Weinhouse, *J. Biol. Chem.* **235**, 2542 (1960); E. Vinuela, M. Salas, A. Sols, *ibid.* **238**, 1175 (1963); D. G. Walker, *Biochim. Biophys. Acta* **77**, 209 (1963).
2. M. Salas, E. Vinuela, A. Sols, *J. Biol. Chem.* **238**, 3535 (1963).
3. C. Sharma, R. Manjeshwar, S. Weinhouse, *ibid.*, p. 3840.
4. C. Gonzáles, T. Ureta, R. Sanchez, H. Niemeyer, *Biochem. Biophys. Res. Commun.* **16**, 347 (1964).
5. H. M. Katzen and R. T. Schimke, *Proc. Nat. Acad. Sci. U.S.* **54**, 1218 (1965).
6. L. Grossbard, M. Weksler, R. T. Schimke, *Biochem. Biophys. Res. Commun.* **24**, 32 (1966).
7. P. McLean, J. Brown, K. Greenslade, K. Brew, *ibid.* **23**, 117 (1966).
8. G. E. Boxer, in G. Weber, *Science* **151**, 479 (1966).
9. V. Lauris and G. F. Cahill, *Diabetes* **15**, 475 (1966).
10. An alternative medium, giving the same results, contained: Hepes buffer, 0.02*M*, *p*H 7.4; 1-cysteine, 30 m*M*; $MgSO_4$, 5 m*M*; K_2SO_4, 15 m*M*. In addition 0.5m*M* glucose was sometimes added to the homogenizing and dialyzing media and to the starch.
11. F. J. Ballard, *Comp. Biochem. Physiol.* **14**, 437 (1965).
12. L. Grossbard and R. T. Schimke, *J. Biol. Chem.* **241**, 3546 (1966).
13. O. H. Lowry, H. J. Rosebrough, A. L. Farr, R. J. Randall, *ibid.* **193**, 264 (1951).

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Psilocybin: Reaction with a Fraction of Rat Brain

Abstract. *Psilocybin, a hallucinogen, formed a blue color with a subfraction of rat-brain mitochondria believed to contain nerve-ending particles. Color formation increased with* p*H, did not require oxygen, and involved a component that could not be solubilized. The effect was not shown by chemically related neuroactive compounds, such as bufotenine and serotonin, and was antagonized by only tyramine or ethylenediaminetetraacetic acid.*

Psilocybin is a hallucinogen whose mode of action is unknown and whose potency is 130 times less than that of LSD-25 (*1*). While investigating the binding of norepinephrine to the crude mitochondrial fraction of rat brain, Herblin (*2*) noted that a blue color developed when psilocybin was present along with the norepinephrine. We now report investigation of the nature of this phenomenon. By use of a method similar to that of De Robertis (*3*), a crude mitochondrial fraction was prepared from rat brain and suspended in 0.32*M* sucrose at (fresh weight) 0.5 g/ml. Samples (1-ml) were each mixed with 1 ml of tris buffer, *p*H 7.4 and 0.15*M*; 0.5 ml of psilocybin, $1.5 \times 10^{-3}M$; and 0.5 ml of water or another reagent where indicated.

Subcellular fractions of the crude mitochondria were prepared by use of sucrose-density gradient of 0.8 ml of 0.7*M*, 0.8 ml of 1.0*M*, 0.8 ml of 1.2*M*, and 1.5 ml of 1.3*M* sucrose. One milliliter of homogenate in 0.32*M* sucrose was layered on top and centrifuged for 45 minutes at 48,000 rev/min in a Beckman L-2 ultracentrifuge with an SW50 head. Five fractions were visible; they were separated with a tube cutter. Microsomal, nuclear, and soluble fractions also were prepared (*3*).

The color, an intense Wedgewood blue, formed only after standing for 16 to 20 hours at 5°C (*p*H 7.4) and was restricted to the precipitate produced by centrifuging for 15 minutes at 3400*g*. Because the blue was in a solid phase, only qualitative statements can be made about its intensity; intensity depended on concentration of psilocybin, being weak at $10^{-4}M$ final concentration and intense at $5 \times 10^{-4}M$. The *p*H was important, for no color was produced at *p*H 6.0; at *p*H 7.4, color was first visible after 8 hours and required about 18 hours for complete development; at *p*H *9* the color was first visible after 1 hour and complete after about 9 hours. Oxygen was apparently not required, for blue formation was not affected by prior passage of N_2 through the crude mitochondria and subsequent incubation with psilocybin under N_2.

Work with brain mitochondria, subfractionated in such a sucrose-density gradient, showed that virtually the only active fraction was that found in 1.2*M* sucrose. This was equally true whether one added the psilocybin to the subfractions or to the crude mitochondria before subfractionation. The 1.2*M* fraction should correspond to De Robertis's fraction C, said to be cholinergic nerve-ending particles; but De Robertis found fraction C to be the richest fraction in serotonin (*4*), while we found most serotonin in the 1.0*M* fraction—0.9 μg per gram of original brain, by the method of Uchida and O'Brien (*5*). Color development by the 1.2*M* subfraction was slower than by crude mitochondria; color was nil at 18 hours and fully developed at 36 hours. No color was developed by the microsomal or soluble fractions. A faint blue color developed by the nuclear fraction may have resulted from incomplete separation. When the crude mitochondria were shaken with water and centrifuged, the precipitate, which should have been free of synaptic vesicles (*6*), showed no loss in ability to develop a blue color with psilocybin. Examination of rat-liver mitochondria, prepared by the same procedure as crude brain mitochondria, showed only a thin light blue on the top of the precipitate after incubation with psilocybin.

We tried to solubilize the color in order to measure its intensity, but the color remained in the precipitate when any of the following reagents was used: butanol, chloroform-methanol (2:1), ether, petroleum ether, hexane, benzene, toluene, Triton X-100 (1 percent in water), acetone, trichloroacetic acid, sodium taurocholate, 0.05*N*

NaOH, and 0.1N HCl. The precipitate was also lyophilized, and attempts to extract the color were fruitless.

None of the following drugs at $5 \times 10^{-4}M$ produced the blue color with crude mitochondria: amphetamine HCl, adrenalin, bufotenine bioxalate, bulbocapnine HCl, chlorpromazine HCl, harmine HCl, LSD 25, or serotonin creatine–H_2SO_4. Formation of the colored complex seems quite specific for psilocybin.

Several compounds were tested for their individual effect on the production of the blue complex. Psilocybin, crude mitochondrial homogenate, and the test compound at the same concentration as the psilocybin ($2.5 \times 10^{-4}M$) were mixed and shaken overnight. Harmine, ouabain, atropine sulfate, *d*-tubocurarine chloride, bufotenine, bretylium tosylate, reserpine, chlorpromazine, sodium pentobarbital, serotonin, and tyrosine had no effect but production of color was about 80 percent inhibited by ethyldiaminetetraacetic acid (EDTA) or tyramine HCl and less inhibited by 2,4-dinitrophenol.

Two questions remain: Has this phenomenon any bearing on the hallucinogenic action of psilocybin? By what mechanism is the color formed? Evidence against a relation to hallucinogenesis is the high concentration required, and the fact that the closely related hallucinogen bufotenine (which has a 5-hydroxyl rather than a 4-phosphate substituent) gives no blue color. However, with respect to the concentration aspect, it is of course possible that the blue color is an extreme manifestation of an effect whose more-subtle consequences, at much lower concentrations of psilocybin, may suffice to affect brain function.

In discussion of mechanisms, two related observations may be considered. Ehrenpreis *et al.* (*7*) found that several catecholamines combine with the highly phosphorylated egg protein, phosvitin, to give a blue color whose formation is blocked by tyramine or EDTA (*8*). Can it be that a phosphate group is needed—in our case provided by the psilocybin? This idea would explain why bufotenine is ineffective in our system. The phosvitin effect increased with *p*H, as did the psilocybin effect. Maas and Colburn (*9*) found that nerve-ending particles and synaptic vesicles contain appreciable amounts of magnesium, copper, and iron; they suggested that the metals in these components form a ternary complex with adenine triphosphate (ATP) and norepinephrine; complex formation is blocked by the chelating agent ethylenediamine. Perhaps in this instance a phosphate requirement is supplied by ATP. The interference reported by us for 2,4-dinitrophenol may result from an effect on endogenous ATP.

There remains a question of whether the formation of blue by psilocybin is connected with norepinephrine binding. Herblin (*2*), after using well-washed crude mitochondrial fraction from rat brain, reported that norepinephrine as well as psilocybin was required for the blue color; but in our unwashed preparation we found no such effect. Yet the interference by tyramine is suggestive, because tyramine is believed to interfere with norepinephrine binding in a special pool from which it is released by nerve impulses (*10*). Finally, psilocybin is reported (*2, 11*) to promote the uptake of norepinephrine by crude rat-brain mitochondria.

The *p*H dependence of the formation of psilocybin blue is compatible with involvement of the unprotonated indole group of psilocybin. The inactivities of bufotenine and serotonin suggest that a phosphate group also is required. It remains to be seen whether additional features of the psilocybin molecule are required for the effect.

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References and Notes

1. L. E. Hollister, *Arch. Intern. Pharmacodyn.* **130**, 42 (1961).
2. W. Herblin, thesis, Cornell Univ., 1965.
3. E. De Robertis, A. Pellegrino De Iraldi, G. Rodriguez De Lores Arnaiz, L. Salganicoff, *J. Neurochem.* **9**, 23 (1962).
4. E. De Robertis, in *Biogenic Amines*, H. E. Himwich and W. A. Himwich, Eds. (Elsevier, New York, 1964), p. 118.
5. T. Uchida and R. D. O'Brien, *Biochem. Pharmacol.* **13**, 725 (1964).
6. E. De Robertis, G. Rodriguez De Lores Arnaiz, A. Pellegrino De Iraldi, *Nature* **194**, 794 (1962).
7. S. Ehrenpreis, H. R. Munson, M. Kaplan, M. Platlby-Lang, in *Proc. Intern. Congr. Biochem. 6th.*
8. S. Ehrenpreis, personal communication.
9. J. W. Maas and R. W. Colburn, *Nature* **208**, 41 (1965).
10. L. T. Potter and J. Axelrod, *J. Pharmacol. Exp. Therap.* **140**, 199 (1963).
11. W. Herblin and R. D. O'Brien, in preparation.
12. Aided by PHS grant GM 07804. We thank Sandoz Co. for donating psilocybin and LSD; Smith, Kline, and French Laboratories for chlorpromazine hydrochloride; and Burroughs Wellcome Co. for bretylium tosylate.

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Abductin: A Rubber-Like Protein from the Internal Triangular Hinge Ligament of Pecten

Abstract. *The rubber-like internal triangular hinge ligament from* Pecten *was studied by light and electron microscopy, x-ray diffraction, and chemical analysis. The ligament is composed of an amorphous protein, abductin. In physical properties abductin is similar to elastin and resilin but distinct by amino acid analysis. It is characterized by high concentrations of glycine and methionine.*

During investigation of the adductor muscles of scallops, a structure that felt like vulcanized rubber was encountered in the hinge. The scallop swims by means of a bellows-like action of the valves, and the internal triangular hinge ligament (ITHL) acts as an antagonist to the adductor muscle. When the valves are closed by contraction of the adductor muscle this ligament is compressed, and upon relaxation of the muscle the elastic spring-like action of the ligament causes the valves to abduct.

Our preliminary investigations and the results of other investigators showed that the hinge ligament has properties similar to those of the other known elastic proteins, elastin and resilin. Trueman (*1*) estimated the modulus of elasticity for the ITHL of *Pecten* to be 3×10^7 dyne/cm^2. Alexander (*2*), correcting for creep, estimated Young's modulus to be 4×10^7 dyne/cm^2. Both investigators measured compression of the ligament. In our measurements of stretch of ITHL with an Instron strain gauge, the modulus is estimated to be 1.25×10^7 dyne/cm^2. Young's modulus for elastin is estimated to be 6×10^6 (*3*) while for resilin it is 4×10^7 dyne/cm^2 (*4*). Alexander concluded from his thermodynamic calculations that the elasticity of the ITHL is true rubber-like elasticity, in that elastic force is associated mainly with entropy changes (*2*). Like resilin and elastin, the ITHL is rubber-like only when it is hydrated and is rigid and leather-like when dry.

We studied the ITHL by light and electron microscopy, x-ray diffraction, and chemical analysis in order to compare the properties of this elastic ligament with those of elastin and resilin.

Ligaments from *Placopecten magellanicus* and *Aequipecten irradians* were dissected away from the valves, and