patterns for variant I and variant II are the result of a mutant A subunit and that the sub-bands in variant II are tetramers composed of combinations of normal A, mutant A (A′), and normal B subunits as shown in Table 1. Thus, variant II is the heterozygote and variant I the homozygous variant. Support for this hypothesis was obtained in dissociation-recombination experiments as originally described (*9*). When a mixture of isolated LDH-1 from a normal baboon and LDH-5 isolated from variant II was frozen and thawed in a medium containing 0.25*M* sodium chloride and 0.1*M* sodium phosphate, *p*H 7.0, the resulting LDH pattern on the starch gel was the same as the pattern of variant-II phenotype pattern. Recombinations of a mixture of isolated normal LDH-1, normal LDH-5, and variant-I LDH-5 could not be made because of lack of adequate amounts of variant-I tissue. The Delta Regional Primate Research Center is attempting to breed male gelada variant II with a female variant II, and it may eventually be possible to test the genetic hypothesis by examination of the progeny.

The incidence of variant LDH types in male and female gelada baboons is not significantly different, although of eight males only one is the type II variant, whereas of 13 females six are type II variants and one is type I variant. The high frequency of LDH variants in the total gelada group (8 out of 21) is in contrast to the rarity of LDH variants (15 out of 5158) in man (*10*). In other catarrhine primates two of 215 studied in our laboratory showed variants in the A subunit of LDH. These two were among 19 Thailand cynomolgus monkeys (*Macaca irus*), but we found no variants among the 107 animals of the same species from the Philippines. Among 17 animals of various Ceboid species we observed a variant LDH pattern of the heterozygous type in one of two Titi specimens.

In the gelada group, the A′ subunit variant is apparently either a case of local genetic drift or an example of more widespread polymorphism of an autosomal LDH-determining gene among the populations of *Theropithecus gelada*.

FRANK N. SYNER
MORRIS GOODMAN
Lafayette Clinic and *Wayne State University School of Medicine, Detroit, Michigan*

References and Notes

1. E. Apella and C. L. Markert, *Biochem. Biophys. Res. Commun.* **6**, 171 (1961); C. L. Markert, *Hereditary, Developmental and Immunologic Aspects of Renal Disease*, J. Metcoff, Ed. (Northwestern Univ. Press, Evanston, Ill., 1962).
2. C. R. Shaw and E. Barto, *Proc. Nat. Acad. Sci. U.S.* **50**, 211 (1963).
3. S. H. Boyer, D. C. Fainer,, E. J. Watson-Williams, *Science* **141**, 642 (1963); W. E. Nance, A. Caflin, O. Smithies, *ibid.* **142**, 1075 (1963); A. P. Kraus and C. L. Neely, Jr., *ibid.* **145**, 595 (1964).
4. E. S. Vesell, *ibid.* **148**, 1103 (1965).
5. This information was provided by Dr. C. W. Hill, Cooperative Research Coordinator of the Delta Regional Primate Research Center.
6. M. M. Dewey and J. L. Conklin, *Proc. Soc. Exp. Biol. Med.* **105**, 492 (1960).
7. A. L. Koen and C. R. Shaw, *Biochim. Biophys. Acta* **96**, 231 (1965).
8. P. J. Fritz and K. B. Jacobson, *Science* **140**, 64 (1963).
9. C. L. Markert, *ibid.*, p. 1329.
10. C. R. Shaw, *ibid.* **149**, 936 (1965).
11. We thank Drs. S. A. Riopelle and C. W. Hill of the Delta Regional Primate Research Center and Drs. C. E. Frohman, A. Koen, C. R. Shaw, and G. Lasker for their cooperation and interest; and H. Yoon for technical assistance. Contributions of materials from the center were supported by PHS grant Fr-00164. Supported by grant GB-1241 from the National Science Foundation.

18 October 1965

Medium for Selective Isolation of Cryptococcus neoformans

Abstract. *A medium has been developed that permits the selective recovery of* Cryptococcus neoformans *from heavily contaminated materials. It employs creatinine as a nitrogen source, diphenyl ($C_6H_5C_6H_5$) and chloramphenicol as mold and bacterial inhibitors, and* Guizotia abyssinica *seed extract as a specific color marker. The medium has proved to be effective in the direct isolation of* Cryptococcus neoformans *from pigeon nests and from the air.*

The development and use of a selective isolation medium for human pathogenic fungi has greatly facilitated diagnostic, ecological, and epidemiological studies (*1*). By incorporating the antibacterial agent chloramphenicol and the antifungal agent cycloheximide into Sabouraud's dextrose agar, pure cultures of most pathogenic fungi are obtainable from heavily contaminated substrata. One of the limitations of this otherwise practical medium lies in its failure to select for *Cryptococcus neoformans*, a yeast that is extremely sensitive to the inhibitory action of cycloheximide.

Staib (*2*) discovered that the purine creatinine, present in bird manure, is readily assimilated by *C. neoformans* and not by other species of the genus *Cryptococcus* or members of the following genera of yeasts: *Bullera, Candida, Debaryomyces, Lipomyces, Pichia, Rhodotorula, Torulopsis*, and *Trichosporon*. One isolate of *Cryptococcus laurentii* proved to be exceptional in that it assimilated creatinine. In addition he observed that a pigment derived from *Guizotia abyssinica* seeds (constituents of canary feed) was selectively absorbed by *C. neoformans*. Staib expressed the hope that these two properties of *C. neoformans* could be utilized in the detection and identification of this important human pathogen. *C. neoformans* grows well on a simple agar medium we made with glucose, creatinine, extract of *Guizotia abyssinica* seed, and chloramphenicol; and the colonies become distinctly brown. However, when plates of this medium were inoculated with material from pigeon nests, the medium was rapidly overgrown with saprophytic molds to such an extent that the *C. neoformans* present could not be isolated. A mold inhibitor was needed. Hertz and Levine (*3*) reported that diphenyl ($C_6H_5C_6H_5$) at a concentration of 100 parts per million suppresses the growth of a large variety of molds but does not inhibit the growth of yeasts. *C. neoformans* was not tested.

We determined that various strains of *C. neoformans* are not inhibited by diphenyl at 100 parts per million. Accordingly, a medium with the following composition was prepared: Glucose, 10.0 g; creatinine, 780 mg; chloramphenicol, 50 mg; diphenyl (dissolved in 10 ml of 95 percent ethanol), 100 mg; *Guizotia abyssinica* extract (*4*), 200 ml; distilled water, 800 ml; and agar, 20.0 g.

Plates of this medium when inoculated with pure cultures of *C. neoformans* supported good growth of pigmented colonies. At 37°C most isolates of *C. laurentii* did not develop; the few isolates that grew on this medium at 37°C did not become brown. *Candida albicans* cultures grew well but remained unpigmented. Several isolations of *C. neoformans* from a series of pigeon nests and from the air have demonstrated the efficacy of the medium.

ALSTON B. SHIELDS*
School of Public Health, University of North Carolina, Chapel Hill 27514

LIBERO AJELLO
U.S. Department of Health, Education, and Welfare, Communicable Disease Center, Atlanta, Georgia 30333

References and Notes

1. L. K. Georg, L. Ajello, M. A. Gordon, *Science* **114**, 387 (1951); C. A. Fuentes, F. Trespalacios, G. F. Baquero, R. Aboulafia, *Mycologia* **44**, 170 (1952); L. K. Georg, L. Ajello, C. Papageorge, *J. Lab. Clin. Med.* **44**, 422 (1954); S. A. Rosenthal and D. Furnari, *J. Invest. Derm.* **28**, 367 (1957).
2. F. Staib, *Zentralbl. Bakteriol. Abt. I Orig.* **186**, 233 (1962); *Z. Hyg.* **148**, 466 (1962); *Mycopathol. Mycol. Appl.* **19**, 143 (1963).
3. M. R. Hertz and M. Levine, *Food Res.* **7**, 438 (1942).
4. Suspend 70 grams of seed powder (pulverized in a blender) in 350 ml of distilled water. Autoclave at a pressure of 10 lb/in.^{2} (1.7 atm) for 10 minutes and filter through gauze. Procedure recommended by Dr. Margarita Silva, Columbia College of Physicians and Surgeons, New York.
5. This preliminary study was carried out under the University of North Carolina—Communicable Disease Center Laboratory Directors' Training Program, which is supported by training grant 5T1GM 567 from the National Institute of General Medical Sciences. Research is being carried out, as part of A.B.S.'s doctoral thesis requirements, at the Communicable Disease Center, Atlnata, Ga.

* Present address: Mycology and Parasitology Section, Communicable Disease Center, Atlanta, Ga. 30333.

29 November 1965

Sorbitol Pathway: Presence in Nerve and Cord with Substrate Accumulation in Diabetes

Abstract. *Glucose, sorbitol, fructose, and inositol are present in peripheral nerve and spinal cord. Marked elevation of these substances occurs in these tissues in mildly diabetic animals. These alterations provide biochemical mechanisms which could be significant in the etiology of diabetic neuropathy.*

The increased conversion of glucose to fructose via the sorbitol pathway has been demonstrated in seminal vesicle and lens of animals with experimental diabetes, and in lens of diabetic patients (*1–3*). The presence of the sorbitol pathway has not previously been described for either peripheral nerve or spinal cord. Stewart *et al.* (*4*) described the presence of fructose in sciatic nerves of normal rabbits by enzymatic and paper chromatographic techniques.

This report shows the presence of sorbitol, fructose, and glucose in peripheral nerve and spinal cord, with marked elevation of these substances in mildly diabetic rats.

Rats of the Sprague-Dawley strain, weighing about 130 to 150 g, were injected intraperitoneally with alloxan in citrate buffer at a dose of 150 mg/kg of body weight. The diabetic animals used had survived 2 weeks without the use of insulin. The animals had constant access to a standard diet with liberal amounts of water and saline. This group showed a consistent 1 to 2 percent content of urinary glucose as measured by glucose oxidase test strips (Combistix, Ames). The animals in this group gained 10 to 20 g during the period of the experiment, while the average gain in the control group was 100 to 150 g. They were killed by a blow to the head followed by decapitation. Blood sugar levels at the time of death were 122 ± 14 mg/100 ml for the control group, and 440 ± 72 mg/100 ml for the diabetic group. Occasional blood sugars of some of the fasted diabetic animals were normoglycemic. The sciatic nerves were isolated and weighed within 5 to 10 minutes and spinal cord within 10 to 15 minutes of death. The tissues were kept at −60°C until used. They were homogenized in zinc sulfate, and precipitated with barium hydroxide. The protein-free filtrate was used for glucose determination by the glucose oxidase method, and sorbitol and fructose were determined by standard procedures (*2*). Part of the protein-free filtrate was evaporated to dryness, and the trimethylsilyl ethers of the sugars present were prepared by the method of Sweeley *et al.* (*5*) and examined by gas liquid chromatography on a column prepared with 15 percent ethylene glycol succinate at 160°C in an argon ionization detection system. The presence of glucose, sorbitol, fructose, and, in addition, inositol, was demonstrated.

Data obtained (Table 1) showed the presence of glucose, sorbitol, and fructose in normal sciatic nerve and spinal cord. There was a marked elevation of these substances in diabetic nerve and cord. Normal sciatic nerve was seen to contain higher levels of these substances than the spinal cord, and the effect of diabetes was more marked. There was a direct correlation, not only between the sorbitol and fructose levels, but also between the glucose and fructose levels in the tissues (correlation coefficient $r = 1$).

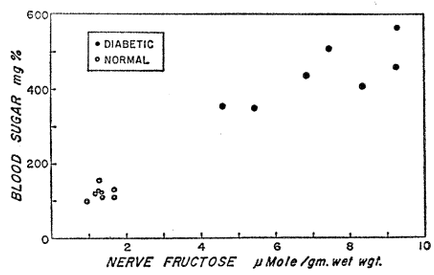

Fig. 1. Relation between blood glucose levels and nerve fructose levels.

Figure 1 shows blood glucose levels plotted against nerve fructose levels, showing a linear increase in the latter with increasing blood sugar levels. The fructose and glucose content of normal rat sciatic nerve, as found by our methods, was similar to that reported by Stewart *et al.* (*4*) for normal rabbit sciatic nerve.

A reasonable estimate of the contribution of extracellular glucose to the levels found in diabetic nerve can be made by assuming a 15 percent extracellular compartment by weight. At a mean blood sugar level of 440 mg/100 ml, the glucose present in the extracellular compartment would not exceed 4.5 μmole/g. The finding of a level of 16.97 μmole/g in diabetic nerve indicated the accumulation of a large, intracellular, free-glucose compartment. A considerable amount of inositol was also found to be present, by gas liquid chromatography, in both sciatic nerve and spinal cord.

The conversion of glucose to fructose via sorbitol has been described for lens and seminal vesicle (Fig. 2).

Kinoshita and Hayman recently demonstrated the presence of aldose reductase in spinal cord (*6*). We have obtained evidence with crude extracts of spinal cord and nerve, indicating the additional presence of polyol dehydrogenase (*7*).

The demonstration of the sorbitol pathway in sciatic nerve and spinal cord, together with the demonstration

Table 1. Content of glucose, sorbitol, and fructose in sciatic nerve and spinal cord of normal and diabetic animals.

Compound	Sugar (μmole/g wet wt. ± S.D.; $N = 12$)					
	Sciatic nerve			Spinal cord		
	Normal	Diabetic	P	Normal	Diabetic	P
Glucose	2.51 ± 1.85	16.97 ± 9.40	<0.001	0.41 ± 0.14	1.70 ± 2.31	<0.02
Sorbitol	1.88 ± 1.02	6.51 ± 1.75	<0.001	1.18 ± 0.47	1.77 ± 0.78	<0.01
Fructose	1.29 ± 0.21	6.51 ± 1.95	<0.001	0.15 ± 0.04	0.96 ± 0.23	<0.001