60 min (os = + 6.1%; p = + 4.3%). After PCPA administration, 5-HT levels decreased 27.4%. After a subsequent administration of 5-HTP, brain levels of 5-HT reached values of 66% and 62% at 30 and 60 min, respectively, as compared with the controls.

*Discussion.* Our data strongly support a direct relationship between the levels of brain 5-HT and EAEP's latency. The administration of 5-HTP, which causes a strong increase of the levels of brain 5-HT, is able to raise the EAEP's latency (onset and peak), both in experiment A and in experiment B. The only difference into the effect of 5-HTP treatment in experiment B seems to be a shorter lasting effect on latency. The fact that the 5-HT content after PCPA treatment cannot be restored to normal level by the following 5-HTP administration, may be responsible for the short duration of the effect. On the other hand, PCPA, which causes a strong decrease of cerebral 5-HT, is able to reduce the EAEP's peak latency. However, the increase of EAEP's latency, caused by the administration of 5-HTP, was higher, in absolute values, as compared with the decrease of EAEP's latency caused by the administration of PCPA. The evoked cortical responses are an objective parameter of the function of these nervous pathways. The lag between the stimuli and the responses are in direct relationship with the induction or the inhibition of the nervous pathways afferent to the interested system. Our results show mainly the inhibitor effect upon the central acoustic pathways by increasing the synthesis of brain 5-HT. The lack of a strong corresponding decrease of EAEP's latency by diminishing the levels of this monoamine may probably be due to the known properties of the CNS, as the conduction velocity of a stimulus does not increase beyond a limit, due to the biophysical properties of the nerve fibres. Such inhibitory action can be exerted through the activation of serotoninergic structures, whose presence has been observed along the nervous acoustic pathways. Finally, it can be hypothesized that feedback mechanisms, through the 5-HT synthesis, regulate the stimuli adaptation in the animals[11].

8 B. K. Koe and A. Weissman, J. Pharmac. exp. Ther. *154*, 499 (1966).
9 G. Curzon and A. R. Green, Br. J. Pharmac. *39*, 653 (1970).
10 E. B. Kern, T. R. Cody and R. G. Bickford, Archs Otolar. *90*, 315 (1969).
11 B. Calogero, Clinica otorino-lar. *5–6*, 291 (1971).

## Lipofuscin accumulation in squirrel monkey spinal cord consequent to protein malnutrition during gestation[1]

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*Summary.* The formation of lipofuscin pigment in the anterior horn cells of the cervical spinal cord has shown in the fetuses and neonates under the extrinsic influence of maternal protein deprivation during the gestation period in the squirrel monkeys.

Although the healthy squirrel monkeys of the genus *Saimiri*, in their adult life, are characterized by the presence of lipofuscin pigment in the various areas of the nervous system, its appearance as early as the fetal or neonatal period has not been observed or described by earlier workers. Lipofuscin pigment, generally referred to as the 'aging pigment', appears in small amounts in the neurons of the adult animals including the humans and increases quantitatively as the organism ages[2,3]. Such an accumulation of lipofuscin pigment in the aging nerve cells could lead to significant changes in normal cellular physiology, which have a direct bearing on important neurophysiological functions[4]. In normal healthy animals, lipofuscin pigment is not observed until after the age of 3 months in rhesus monkeys[5] and 2 or 2.5 years in hogs and dogs[6,7].

We report that typical lipofuscin pigment, histochemically identical to the aging or 'wear and tear' pigment[8], accumulates in the anterior horn cells of the spinal cord of the squirrel monkeys born to mothers given protein deficient diets during most of the gestation period. The day of conception was determined by physical palpation of the uterus on a fixed day every week and cytologic examination of the vaginal smears. Before conception, the squirrel monkeys weighed 620–710 g. Beginning at day 35 of conception, the critical period of rapid brain growth, 59 squirrel monkeys were maintained in 2 groups picked up at random from the colony on ad libitum high protein (25% calories from casein as a protein source) and low protein (8% calories from proteins) regimens with 40% calories from a fat source and supplemented with vitamins and minerals. The higher level of calories from fat appears to improve the taste of food and helps increase the total caloric intake[9,10]. Fetuses from 2 animals in each group were removed by cesarian section at 115 days and 140 days of gestation. Together with the neonates these constituted the 28 animals we investigated. Whereas the healthy neonates weighed about 115 g, the malnourished neonates weighed around 80 g. The caloric intake of the mothers in high and low protein group ranged from 80 to 120 calories per animal per day. No statistically significant difference in the total caloric intake was observed in the 2 groups, except during the first 30 days in the low protein group. The average intake

1 Acknowledgments. This work was supported by US PHS grants RR-00165 HD-06087 from National Institute of Health. The technical assistance of Miss Judie Seubert is greatly appreciated.
2 E. A. Porta and W. S. Hartroft, in: Pigments in Pathology. Ed. M. Wolman. New York, Academic Press 1969.
3 G. H. Bourne, Prog. Brain Res. *40*, 187 (1973).
4 T. Samorajski, J. R. Keefe and J. M. Ordy, J. Geront. *19*, 262 (1964).
5 K. R. Brizzee, J. M. Ordy and B. Kaack, J. Geront. *29*, 366 (1974).
6 D. V. M. Whiteford and R. Getty, J. Geront. *21*, 31 (1966).
7 A. Few and R. Getty, J. Geront. *22*, 357 (1967).
8 B. L. Strehler, Adv. Geront. Res. *1*, 343 (1964).
9 S. L. Manocha, Lab. Anim. Sci. *26*, 649 (1976).
10 S. L. Manocha and J. Long, Primates *18*, in press.

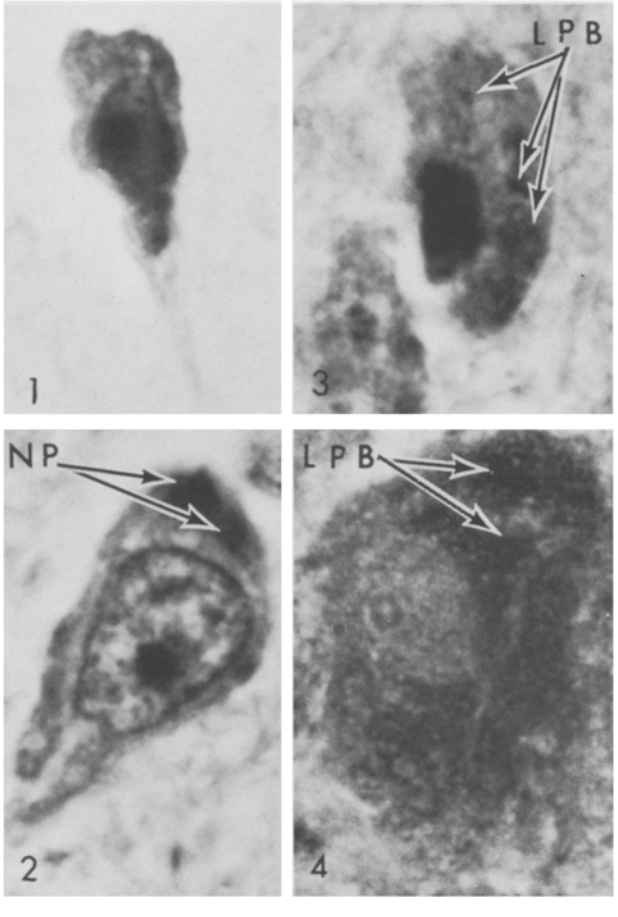
during this period was around 50 calories per animal per day. This appears to be an adjustment period for the animals to get used to a low protein diet.

Besides the routine hematoxylin-eosin and Masson's trichrome methods, several histochemical tests for the demonstration of lipids (e.g. Sudan black B, acid hematin, Sudan IV, Oil red 0, Nile blue sufate), carbohydrates (periodic acid schiff (PAS) with controls), nucleic acids (Feulgen, methyl green/pyronin G; MG/PG), proteins and enzymes (alkaline and acid phosphatases, esterases and oxidative enzymes) were used on formalin-fixed and fresh frozen sections[11]. In addition Millon's test for tyrosines, coupled tetrazolium reaction (with dinitrofluorobenzene, performic acid and benzoylation controls) for tyrosine, tryptophan and histidine were performed. Ferric-ferricyanide, dihydroxy-dinaphthyl-disulfide (DDD) and alkaline tetrazolium reactions were employed for SS and SH groups. Also tried were long Ziehl-Neelsen (Z-N), indophenol, Schmorl's and Dam's method for the specific demonstration of lipofuscins[12].

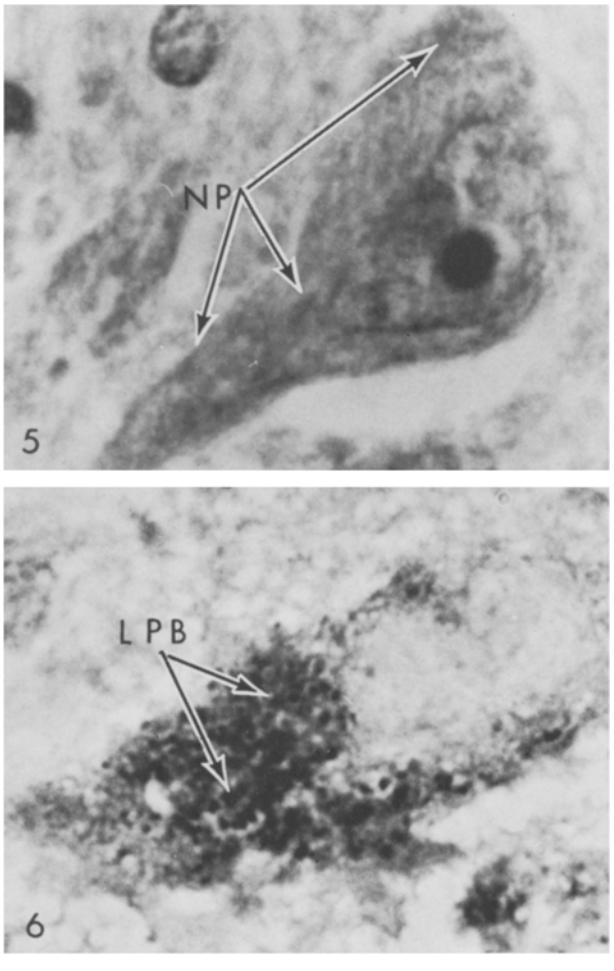
The anterior horn cells of the fetuses and neonates of the control animals did not have lipofuscin pigment. A few neurons in 2 out of 10 neonates studied, showed a particulate or 2 with the characteristic appearance of lipofuscin (figures 1, 2 and 5). In the experimental animals the anterior horn cells of the 115-day-old fetuses showed the presence of a few lipofuscin bodies in the cytoplasm (figure 3), but in 140-day-old fetuses, significant increase in their number was clearly discernible (figure 4). In the neonates born to malnourished mothers, the lipofuscin accumulation in the cytoplasm of the motor neurons of the spinal cord is clearly evident (figure 6). Most often the lipofuscin bodies are scattered all over the cytoplasm but in 4 neonates in the malnourished group, a number of neurons show 1 or 2 polar aggregations of the pigment, generally at the base of the apical dendrite. In such a case, the pigment bodies can be observed in the dendritic process for some length. The pigment we observed consisted of pale-yellow and light to dark brown bodies. Often it also appeared as duplex structures of 0.5–1.3 μm diameter, each with a thin, irregular, light to dark brown cortex enclosing a medulla containing a yellow refringent substance (figures 4 and 6). Similarity of this lipofuscin to the 'aging' pigment was demonstrated histochemically. The lipofuscin bodies were resistant to various fat solvents, viz. acetone, ethanol, chloroform and pyridine. They stained intensely with Sudan black B, periodic acid schiff and long Ziehl-Neelsen stains, mod-

11 A. G. E. Pearse, Histochemistry, Theretical and Applied. The Williams and Wilkins Company, Baltimore I, 1968, 1972.

12 S. P. Sharma, Experientia *17*, 125 (1961).




Figures 1 and 2 (fig. 1, PAS; fig. 2, MG/PG) represent anterior horn cells of the spinal cord from fetuses removed by cesarian section from healthy animals at 115 and 140 days gestation respectively, showing only Nissl patches (NP). Figures 3 and 4 (fig. 3, MG/PG; fig. 4, Z-N) from the fetuses of the experimental animals showing small and substantial amounts of lipofuscin pigment bodies (LPB), respectively.




Figures 5 and 6. Fig. 5 shows an anterior horn cell from the spinal cord of a healthy neonate with no lipofuscin pigment bodies. This is in contrast to large amounts of lipofuscin pigment (LPB) observed in an anterior horn cell of a neonate born to a malnourished mother as shown in Fig. 6 (Z-N).

erately with Nile blue sulfate and methyl green/pyronin G and were negative to the acid hematin test. The pigment reduced ferricyanide to ferrocyanide in Schmorl's test and was strongly positive in the indophenol and chrome-alum hematoxylin reactions. In addition, fat peroxides and traces of several amino acids were recorded. The coupled tetrazonium reaction (accompanied by its controls), and the dihydroxy-dinaphthyl-disuphide (DDD) and alkaline tetrazolium tests were moderately positive, showing the presence in the lipofuscin bodies of SH and SS groups. The pigment was also active for several enzymes, especially acid phosphatase and AS-type non-specific esterase. Our characterization of the lipofuscin pigment thus agrees with that of the aging pigment described by several workers[13-17].

The presence in the lipofuscin pigment of large amounts of lysosomal enzymes suggest that lysosomes are involved in its formation. The concept of the lysosomal origin of lipofuscin is supported by several workers who believe that the lipofuscin bodies may represent true lysosomes and be formed when lipids and lipo-protein precursors undergo peroxidation and polymerization of polyunsaturated fatty acids[18-21].

13 N. M. Sulkin and A. Kuntz, J. Geront. *1*, 533 (1952).
14 T. Samorajski, J. R. Keefe and J. M. Ordy, J. Geront. *19*, 261 (1964).
15 T. Samorajski, J. M. Ordy and J. R. Keefe, J. Cell Biol. *26*, 779 (1965).
16 K. Nandy, Acta neuropath. *19*, 25 (1971).
17 S. P. Sharma and S. L. Manocha, Mech. Aging Develop. *6*, 1 (1977).
18 N. M. Sulkin, J. Geront. *7*, 533 (1952).
19 E. Essner and A. B. Novikoff, J. biophys. biochem. Cytol. *9*, 773 (1961).
20 V. Brunk and J. L. Ericsson, J. Ultrastruct. Res. *38*, 1 (1972).
21 V. N. Karnaukhov, Exp. Cell Res. *80*, 479 (1973).

## Uptake of glucose-C$^{14}$ and fructose-C$^{14}$ by buffalo spermatozoa in extenders during cold storage

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*Summary.* Buffalo spermatozoa exhibit insignificant uptake of glucose-C$^{14}$ and fructose-C$^{14}$ when semen samples were preserved in cold for 96 h in 2 extenders. Incorporation of C$^{14}$ in spermatozoa, TCA precipitable proteins and soluble sugar phosphates was either equal or less in semen samples preserved in cold. CAW appeared to be a better extender than SKMEY as revealed by sugar uptake in cold.

The survival of spermatozoa in the environment of an extender for a reasonable period during cold storage is a pre-requisite for its use in artificial insemination[2]. The conventional egg-yolk citrate (EYC) diluent used for bull semen[3] is less effective for buffalo semen preservation[4], and the buffalo spermatozoa survived for a shorter time than bull spermatozoa[4-6]. Recently, it has been reported from this laboratory[7,8] that citric acid whey (CAW) is a superior diluent for the preservation and freezing of buffalo semen. The preservation characteristics of buffalo semen in this extender has also been reported[9]. The leakage of acrosomal enzyme like hyaluronidase is relatively much less in buffalo spermatozoa preserved in CAW in cold and frozen condition[10]. In the light of these observations, the uptake of glucose-U-C$^{14}$ and fructose-U-C$^{14}$ by buffalo spermatozoa extended in CAW and in skim-milk egg yolk[11] (SKMEY) had been studied when preserved in cold. This report presents these data which indicate relatively less uptake of these sugars by the sperm when preserved in CAW.

*Materials and methods.* Ejaculated semen was collected by means of an artificial vagina[12] from 6 Murrah buffalo bulls of the institute herd. 2 successive ejaculates were

1 Acknowledgment. We are indebted to Dr D. Sundaresan, Director, for his kind encouragement in this study.
2 J. P. Maule, The Semen of Animal and Artificial Insemination. Commonwealth Agr. Bureaux, England, 1962.
3 P. H. Phillips and H. A. Lardy, J. Dairy Sci. *23*, 399 (1940).
4 P. Bhattachara, in J. P. Maule[2].
5 D. R. Gokhale, Indian vet. J. *35*, 97 (1958).
6 C. K. Rao, Indian vet. J. *35*, 97 (1958).
7 N. C. Ganguli, M. Bhosrekar and J. Stephen, J. Reprod. Fert. *35*, 355 (1973).
8 N. C. Ganguli, Indian Dairyman *26*, 243 (1974).
9 M. Bhosrekar and N. C. Ganguli, J. Reprod. Fert. *48*, 215 (1976).
10 N. C. Ganguli, in preparation.
11 N. S. Tomar and R. N. Desai, Indian J. Dairy Sci. *14*, 61 (1961).
12 H. A. Herman and F. W. Madden, The Artificial Insemination of Dairy Cattle. A Handbook and Laboratory Manual. Lucas Brothers, Columbia 1953.

Table 1. Incorporation of glucose-C$^{14}$ and fructose-C$^{14}$ into semen components during cold preservation in extender

| Radioactive sugar substrate | Extender used | Storage period (h) | Specific activity in fractions | | | |
|---|---|---|---|---|---|---|
| | | | Spermatozoa | Proteins (TCA precipitate) | Sugar phosphates Barium soluble | Barium insoluble |
| Glucose-U-C$^{14}$ | CAW | 0 | 8.19 | 0.54 | 10.21 | 7.76 |
| | | 96 | 2.55 | 0.55 | 2.35 | 7.62 |
| Fructose-U-C$^{14}$ | CAW | 0 | 1.72 | 0.64 | 4.01 | 9.93 |
| | | 96 | 1.48 | 0.58 | 2.67 | 11.78 |
| Glucose-U-C$^{14}$ | SKMEY | 0 | 0.70 | 0.55 | 3.54 | 12.57 |
| | | 96 | 0.88 | 0.37 | 2.80 | 5.88 |
| Fructose-U-C$^{14}$ | SKMEY | 0 | 1.30 | 0.23 | 2.40 | 9.74 |
| | | 96 | 0.41 | 0.14 | 2.51 | 7.52 |