into an Rf 11 band for 106 µg and Rf 23 for 212 µg. Abdominal protein resolved an Rf 23 band from 50 µg applied and 33 for 100 µg, 57 for 200 µg and 66 for 300 µg. These abdominal bands illustrated a significant increase in mobility as compared to the other extracts. Deletion of the Triton from the gels resulted in a fragmentation of the major fraction (Figure 2a).

Electrophoreted antennal extracts in 3, 5 and 7% acrylamide gels, containing 0.6% Triton X-100, are illustrated in Figure 2b. As the acrylamide concentration was reduced, the Rf value of the major band was only slightly increased; however, the density of this band was progressively reduced, while the density of a proteinaceous band at the glycine front was progressively increased. 100 µg of antennal extract resolved into an Rf 26 band with the 3% acrylamide, 21 with the 5% and 19 with the 7%.

Under the above conditions, Triton X-100 aggregated, and maintained, most of the solubilized proteins in one heterogeneous fraction. The sieving effects of the different acrylamide concentrations remained high. This indicated a cross-linked proteinaceous structure, with subunit break-off running at the glycine front.

Electrophoresis of 0.6% Triton X-100 in 0.9% NaCl solubilized extracts in pH 7.1 gels (with or without Triton incorporation) and pH 7.1 reservoir buffers resolved several proteinaceous fractions. Employment of different acrylamide concentrations produced distinctive sieving effects on certain of these electrophoreted bands (Figure 2c). When the radiolabelling experiments of FERKOVICH and NORRIS[4] were repeated, except at pH 7.1, label was incorporated into band Rf 81 in 3.5% acrylamide, bands Rf 77 and 56 in 7% acrylamide and band Rf 21 in 14% acrylamide (Figure 2c)[7]. These observations demonstrated an apparent sieving action by the denser acrylamide gels on the proteins binding the labelled feeding inhibitor, menadione$^{14}$C.

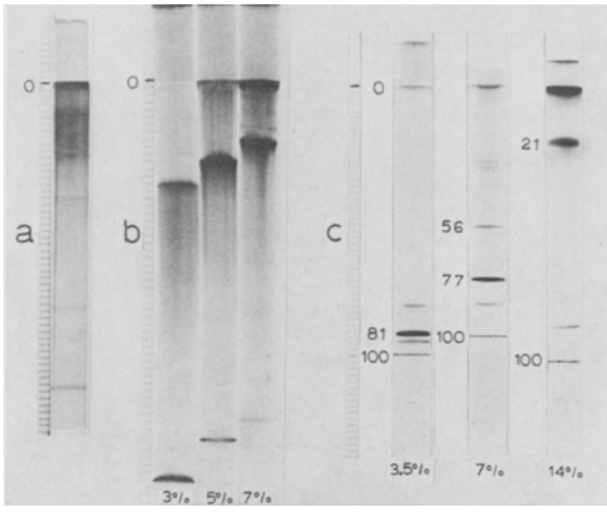


Fig. 2. Electrophoreted Triton-solubilized antennal proteins a) in gel not containing Tritton X-100, pH 8.5; b) on different concentrations of acrylamide, pH 8.5; and c) in different concentrations of acrylamide, pH 7.1.

Resuspension of pellets in different concentrations of Triton X-100 resulted in altered protein solubilization. Folin analysis[6] and electrophoresis of samples indicated that a 1% solution of Triton in 0.9% NaCl solubilized less than 1/2, and a 5% solution solubilized less than 1/4 as much protein as did the 0.6% Triton extraction.

SDS electrophoresis has become an established means for the determination of protein molecular weights[8]; however, addition of SDS to our obtained proteinaceous extracts, as yet, has produced no banding with SDS electrophoresis.

Although Triton X-100 is commonly employed in the solubilization of pellet-bound proteins, our findings illustrated and emphasized certain effects which Triton can have on protein solubilization and fractionation. Experimental parameters regarding Triton treatment and use must be accurately defined and controlled to insure reproducible, meaningful results[9].

*Resumen.* Un agregado de proteínas solubilizadas en Triton X-100 fue resuelto como una banda por electroforesis a un pH de 8.5. En un pH de 7.1 estas fracciones no demuestran el efecto de agregación. Solamente electroforesis con *Tris*/glicina como amortiguador y concentraciones del detergente menor de 0.6% dieron resultados.

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[6] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[7] G. SINGER and D. M. NORRIS, unpublished data.
[8] D. GOSPODAROWICZ, Endocrinology *90*, 1101 (1972).
[9] This research was supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison; and in part by funds from the Acme Chemical Co., Milwaukee (Wisconsin, USA).

## Occurrence of Vesicles in Rabbit Seminal Plasma

The fertilizing capacity of rabbit spermatozoa recovered from the uterus of a doe is inhibited by a fast sedimenting component in seminal plasma from intact and vasectomized bucks that was isolated recently following centrifugation on sucrose density gradients[1]. Rabbit seminal plasma had been previously postulated to possess a factor of macromolecular dimensions capable of reversibly blocking sperm fertilizing capacity, when it was observed ultracentrifugation removed decapacitation activity in a few hours[2]. This report shows the inhibitor fraction consists of numerous small vesicles and, in addition, that another rapidly sedimenting seminal plasma fraction of higher density also contains vesicles.

Seminal plasma was collected with an artificial vagina from bucks of mixed strains, having proven fertility. Fresh semen samples with good sperm motility were centrifuged at $1000 \times g$ for 30 min and the seminal plasma aspirated. Rapidly sedimenting components were recovered from this fluid by employing discontinuous

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[2] J. M. BEDFORD and M. C. CHANG, Am. J. Physiol. *202*, 179 (1962).

density gradients containing layers of 20, 40 and 60 or 80% (w/v) sucrose with 0.15 *M* KCl and 0.01 *M Tris*, pH 7.4. After centrifugation at 25,000 rpm for 9 h in a Spinco SW 27 rotor at 5 °C, the cellulose nitrate centrifuge tube containing the sucrose density gradient preparation was punctured at the base and 1 ml fractions collected in test tubes below. The absorbance of these fractions at 270 nm was determined in a Beckman DU spectrophotometer. Fractions corresponding to peaks were pooled and dialyzed in Visking tubes against approximately 1000 vols. buffer (0.15 *M* KCl, 0.01 *M Tris*, pH 7.4). The 2 rapidly sedimenting fractions obtained were sedimented at $168{,}000 \times g$ for 2.5 h in a titanium 50 rotor (Spinco). Enough material was centrifuged to form a visible pellet (diameter > 0.3 cm) at the base of a centrifuge tube. The pellet was fixed immediately with 2% $OsO_4$, subsequently embedded in Epon (Ladd Industries), sectioned with a glass blade microtome, stained by 20% uranylacetate and 0.5% lead citrate and finally examined in a Zeiss electron microscope (model EM 9S2). Sections were also prepared of spermatozoa flushed from the cauda epididymis of a buck by medium 199 (Difco) and then sedimented at $12{,}000 \times g$ for 10 min in a Sorvall RCB2 centrifuge at 5 °C to form a sperm cell pellet.

Fast sedimenting entities in rabbit seminal plasma are arrested in the density gradient shown by Figure 1 at the 40 and 60% sucrose zones. 2 visibly cloudy bands can be seen and they are designated Fr I and II. Seminal plasma remaining at the top of the gradient (above the 20% sucrose zone) is not turbid. Fractions I and II had densities of 1.20 and 1.16 g/cm³, respectively, in a linear 20–60% (w/v) sucrose gradient, centrifuged at 25,000 rpm in an SW 27 rotor for 48 h at 5 °C; fraction densities were measured with a refractometer (Bausch and Lomb). Schlieren patterns observed for Fr I and II in an analytical ultracentrifuge (Spinco, model E), operated at 33,450 rpm at 20 °C, showed single peaks with mean sedimentation coefficients of 250S for Fr I and 85S for Fr II.

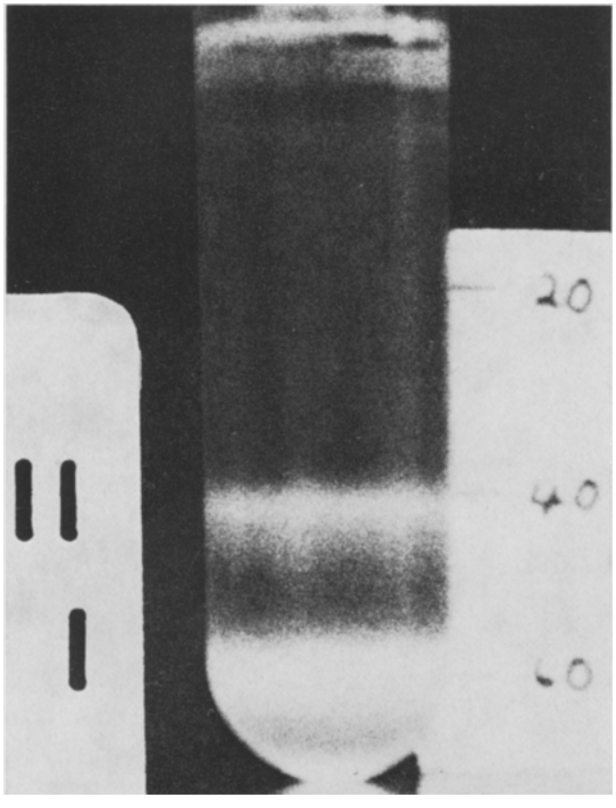


Fig. 1. Shows location of bands of rapidly sedimenting components in rabbit seminal plasma on a discontinuous sucrose density gradient following ultracentrifugation. Seminal plasma (10 ml) was pipetted on top of the density gradient that contained zones of 3 ml 60% sucrose, 9 ml 40% sucrose and 10 ml 20% sucrose in 0.15 *M* KCl and 0.01 *M Tris*, pH 7.4, and then centrifuged. Fractions I and II appear as cloudy bands that are arrested by the 40 and 60% sucrose zones at the locations indicated.

Electron micrographs of sections cut after sedimentation of Fr I and II reveal them to consist of numerous small, round vesicles (Figure 2). A trilaminar membrane boundary can be seen that is estimated to be 83 Å thick. Fraction I vesicles had a mean cross-sectional diameter of 607 Å and Fr II had a diameter of 509 Å, based on samples of nearly 300 vesicles each. About 10% of the vesicles in each preparation had unusually large diameters (> 800 Å) and aggregation during sedimentation could have contributed to their presence. The mean vesicle diameters yield spherical volumes of about $1.2 \times 10^{-16}$ and $0.7 \times 10^{-16}$ cm³. Vesicle sizes correlate broadly with their rate of sedimentation and are consistent with 'molecular weight' values of several million daltons. It may be estimated that rabbit seminal plasma has approximately $10^{14}$ vesicles/ml; they are evidently more numerous than spermatozoa in semen by several orders of magnitude.

A peak corresponding to Fr II was not observed in schlieren patterns formed during ultracentrifugation of cauda epididymal fluid (diluted 1:3 with medium 199), in contrast Fr I occurred at an estimated concentration of 2 mg/ml. Figure 3 shows an epididymal spermatozoa with Fr I-like vesicles on its surface. Their presence on the sperm cell is associated with the loss of membranes (Figure 3). Conceivably these vesicles are formed from spermatozoan membranes during sperm cell breakdown in the epididymis. Fr I vesicles were found to be virtually absent from rabbit testis. Since Fr II vesicles are present in seminal plasma from vasectomized male rabbits, they are clearly of non-spermatozoan, post-epididymal origin. These vesicles are present in the seminal vesicle and may arise from it.

Seminal plasma from bull and man, in addition to that from rabbits, have 2 classes of vesicles that are arrested by 40 and 60% sucrose zones in discontinuous density gradients sedimented under the conditions described. Seminal plasma from these 3 species are known to decapacitate rabbit sperm obtained from the uterus of a doe[3]. Moreover, Fr II was previously identified as possessing decapacitation activity[1]. Participation of vesicles in decapacitation suggests it may be a membrane associated phenomenon. How these vesicles serve to stabilize the sperm cell plasma membrane and prevent prefertilization vesiculation in the acrosomal region[4] is a subject of current interest[5].

*Zusammenfassung.* Es konnte gezeigt werden, dass die 2 schnell sedimentierende Komponenten, isoliert aus Kaninchensamenflüssigkeit von verschiedenen Zonen eines unterbrochenen Sucrosegradienten in elektronen-

[3] M. C. Chang, Nature, Lond. *179*, 258 (1958).

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[5] Results contained in this communication were presented at a National Institutes of Health workshop, Bestheda, Maryland, USA, January 12, 1973. The electron micrographs were kindly prepared by Mr. K. Bedigian. Financial support was received from N.I.H. Contract No. I–HD–3–2781.

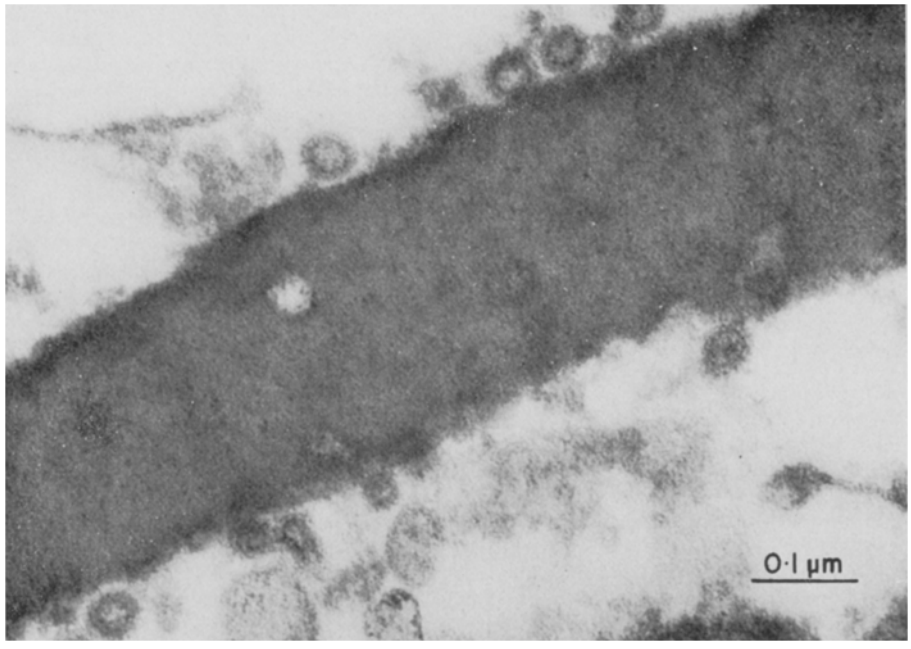


Fig. 3. An electron micrograph of an epididymal spermatozoon showing Fr I-like vesicles.

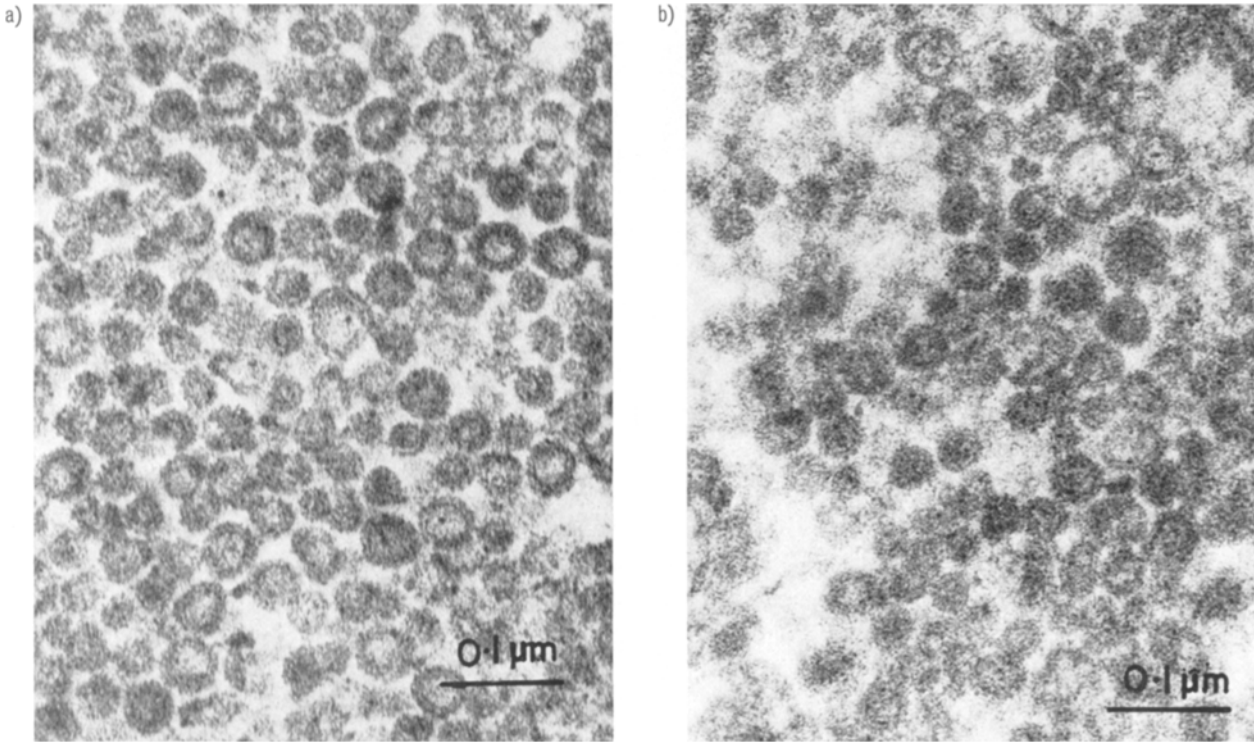


Fig. 2. Electron micrographs from ultracentrifugal sediments of isolated rabbit seminal plasma fractions. These preparations were fixed in $OsO_4$ and stained with uranylacetate and lead citrate. a) Fr. I, b) Fr II.

mikroskopischen Aufnahmen vesikuläre Strukturen darstellen. Die langsamer sedimentierende Komponente (85S) hat einen kleineren Durchmesser (507 Å) und ist weniger dicht (1.16 g/cm³) als die schneller sedimentierende Komponente mit einem durchschnittlichen Durchmesser von 609 Å und einer Dichte von 1.20 g/cm³. Die Dekapazitationsaktivität in Samenflüssigkeit kommt beiden zu und nicht nur wie bis jetzt angenommen wurde, der 85S Komponente. Zwei verschiedene Vesikeltypen wurden auch in der Samenflüssigkeit von Mensch und Stier gefunden.

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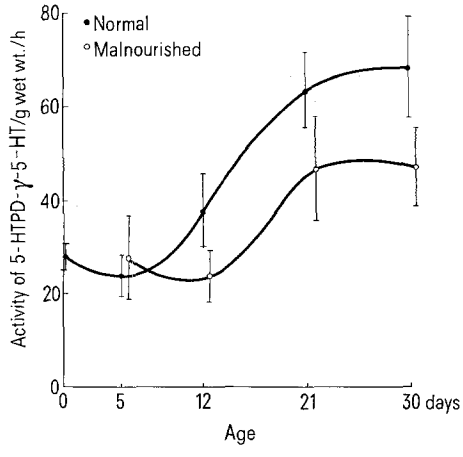
## Developmental Pattern of the Serotonin Synthesizing Enzyme in the Brain of Postnatally Malnourished Rats

Study of the metabolism of brain biogenic amines in animals malnourished from birth represents an opportunity to evaluate the effects of malnutrition on a biochemical parameter closely related to neuronal function during development and maturity. 5-Hydroxy-tryptamine (5-HT) acts in the brain as a neurotransmitter and seems to participate in the integration and control of psychoemotional behaviour[1-6]. We have therefore considered it of importance to study the metabolism of 5-HT in the brain of normal and malnourished developing rats. The activity of the brain enzyme 5-Hydroxyrtyptophane-Decarboxylase (5-HTPD) which synthesizes 5-HT was measured as well as the concentration of this amine. The results will be outlined here.

*Methods.* Albino rats, originally Wistar strain, were malnourished from birth with the method used by Widdowson and Kennedy[7]. Newborn rats were redistributed within 12 h of birth into litters of 6 and 16 per mother, the former were considered controls and the latter malnourished. On day 21 the young were separated from the mother in individual cages and fed Purina chow ad libitum (control group) or 50% of the normal caloric requirements (malnourished group). Water was offered ad libitum to all animals. Body and brain weight curves were determined in both groups. Animals were killed by decapitation on days, 5, 12, 21, and 30, the brain was dissected (cerebellum, pineal and olfactory bulbs were not included), weighed, and homogenized in 0.25 *M* cold sucrose or 0.1 *N* HCl. For the estimation of 5-HTPD-activity the method of Kuntzman et al.[8], was used. For measuring serotonin concentration, the method described by Snyder et al.[9] was employed.




Activity of 5-Hydroxytryptophane-decarboxylase in the brain of rats malnourished from birth and normal controls. Each point represents the mean activity from 3 to 14 samples, from at least 3 different litters ± S.E. The enzyme activity is expressed as μg of 5-HT/g of wet weight/1 h.

*Results and discussion.* Deficits in body and brain weight in malnourished animals were, at 30 days of age, of the order of 40% and 15% respectively as compared with the controls. The developmental curve of the activity of 5-HTPD in the whole brain of rats from both groups studied is shown in the Figure. In the control group the enzyme curve shows a decrease in activity between birth and the 5th day, increasing thereafter up to the 21st day, with a lower rate of increase between days 21 and 30. 5-HTPD activity in the brain of malnourished rats showed a tendency to be lower than in controls, being significantly lower on day 21 ($p < 0.05$), calculated on the basis of dry weight (Table I). The decrease in enzyme activity observed on the 5th day in the controls was observed on the 12 day in the brain of malnourished

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Table I. Activity of 5-Hydroxytryptophane-Decarboxylase (μg of 5-HT/g dry weight/1 h ± S.E.)

| Age | 12 days | 21 days[a] |
|---|---|---|
| Normals | 553.9 ± 62.4 | 1061 ± 90.4 |
| Malnourished | 369.8 ± 126.9 | 821.7 ± 65.2 |

[a] $p$ 0.05.

Table II. Mean concentrations of 5-Hydroxytryptamine, ng/g wet weight, in the brain of Normal (N) and Malnourished (M) rats ± S.E.

| | Age in days | | | | |
|---|---|---|---|---|---|
| | 0 | 5 | 12 | 21 | Adults |
| N | 345 ± 7.7 | 138 ± 20.0 | 277 ± 10.7 | 352 ± 42.0 | 523 ± 33.1 |
| M | — | 151 ± 12.6 | 345 ± 30.2 | 307 ± 66.2 | — |