Table III.

Comparison	D.F.	t	P
A–B	48	0,053	>95%
B–C	48	0,100	>90%
A–C	48	0,021	>98%

those of the vertebrates Nissl bodies, such as in *Helix pomatia*[10] and in *Lymnaea stagnalis*[11]. In the light of current knowledge, the intensity and arrangement of the basophilia reflect more a physiological stage than a constant and conspicuous feature. Moreover, there is evidence that basophilia seems to have variations in correlation with the phasic periodical activity of adductor muscles in *Anodonta cygnea*[12]. By cytophotometric analysis, it was also found that concentration of cytoplasmic RNA is equivalent to the total nucleic acids (DNA + RNA) within the nucleus[12]. Typical images of 'chromatolysis' were found at random in the ganglia studied, as is shown in Figures d) and e) while others show atypical distribution of the chromatin (Figure f). Both 'anomalies' did not exceed 2% of the neurons.

Finally, another point to be stressed is the hypothesis, advanced by many authors[13–15], related to the poliploid nature of molluscan neurons. As is well known, the degree of poliploidy causes nuclear (and total) enlargement of the cell, and a ratio 1:2:4 among the types can be expected[7]. Nuclear sizes, as well as maximal and minimal cell diameters, were studied related to this ratio, but since these data show a significative correlation, only the statistical treatment for the maximal cell diameter will be given here. This relationship was tested making a t-test of the null hypothesis of the difference between the mean of a given diameter and the mean of the other times the assumed coefficient ($\bar{x}_1 - n\bar{x}_{1+k} = 0$). For this purpose the pooled variance was the same of the analysis of variance. All values obtained (see Table III) show that the null hypothesis cannot be disproved, in consequence it is possible to assume safely that these differences are not significant, and the 1:2:4 ratio can be statistically proved. Assuming a generalized elipsoidal shape for these neurons, their volumes were estimated in about 3.2×10^4 μm^3 for the type C, 2×10^5 μm^3 for the type B and 2×10^6 μm^3 for the type A. These data gave a ratio among the volumes of the types of 1:6.3:63.

The presence of at least 2 different cell populations was reported in *Aplysia californica* after the measurement of the DNA content in central giant neurons, by a fluorescent method[16]. The enormous amount of DNA found per nucleus suggest that a part or all the genome replicates many times[16]. The occurrence of poliploidy in mammalian central neurons has also been reported[17]. However, no definite conclusions on the possibility of a functional significative increment of the DNA in such neurons appear well established[18].

Resumen. Se realiza en la masa ganglionar ventral del molusco *Cryptomphallus aspersa* (Gasteropoda, Pulmonata) la caracterización morfologico-estadística de las neuronas centrales y células gliales. Se establece la existencia de tres tipos neuronales, las características de su basofilia y la relación entre los tipos, lo que se discute en relación a los hallazgos obtenidos en otras especies por diversos autores.

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Sialic Acid in Human Cancer

The relative proportion of the two main sialic acids, N-acetyl-neuraminic acid (NANA) and N-glycolyl-neuraminic acid (NGNA), varies considerably in different mammalian species; but man seems to be unique in that only NANA has been found[1]. In human cancer, quantitative changes in sialic acid have been reported. Analyses of several types of human cancer tissues revealed that the area of malignancy contained almost twice as much sialic acid as the normal areas of the same tissues[2].

Qualitative changes in sialic acids have been found in experimental cancer. Reports have indicated that although normal rat liver does not contain NGNA, this form of sialic acid was observed in hepatomata induced by the feeding of *p*-dimethylaminobenzene[3] and in several rat ascites hepatoma[4].

These specific chemical changes associated with malignancy, and the recent discovery of NGNA[5] in Hela S_3 cells (a line of cells of human cancer origin) prompted this qualitative and quantitative study of the sialic acids in human cancer tissue.

Materials and methods. Human cancer tissues were obtained from the autopsies of cancer victims while normal

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controls were obtained from the autopsies of non-cancer patients. The tissue was first homogenized in 0.1 *N* sulfuric acid (1:7 w/v) with a Waring Blendor, and then the acid homogenate was heated at 80 °C for 1 h in order to liberate the ketosidically bonded sialic acids[6]. After removal of the sulfuric acid as insoluble barium sulfate, the hydrolysate plus 3 water washings of the sediment were passed successively through volumns (1 × 23 cm) of Dowex 50 W (H^+) and Dowex I (formate). The columns were washed with distilled water, and the sialic acid was eluted from the Dowex I (formate) column with 0.3 *N* formic acid. The effluent was collected in 10-ml fractions with an automatic fraction collector. All sialic acid positive fractions were pooled and lyophilized to remove formic acid, and the residue was dissolved in distilled water. The sialic acid content was then determined by the thiobarbituric acid method[7] with crystalline NANA as the standard. *Identification* of NANA and NGNA was accomplished by paper chromatography[8] on Whatman No. 1 Chromatography paper using *n*-butanol-*n*-propanol-0.1 *N* HCl (1:2:1) and the sialic acids were detected with the thiobarbituric acid spray-reagent[9]. A mixture of standard NANA and NGNA was run on the same paper, next to the sialic acid isolated from tissues.

Results and discussion. The chromatographic analyses of the sialic acids isolated from malignancies found in 4 different organs – pancreas (adenocarcinoma), liver (metastasis from pancreatic adenocarcinoma), skin (squamous cell carcinoma) and paratracheal lymph node (metastasis from skin melanoma), revealed the presence of NANA but no evidence for the presence of NGNA was ever obtained.

The sialic acid content of the cancerous tissues was considerably elevated. The values obtained in the analyses of pancreatic tissues (expressed as mg of NANA per 100 g of fresh tissue) were 45.8 for normal pancreas, 187.0 for the adenocarcinoma and 75.5 for the liver metastasis of pancreatic adenocarcinoma.

The results of the experiments described indicate that, unlike the situation observed in the rat, both normal and cancerous human tissues contain only NANA.

Our quantitative studies support previous findings of BARKER et al.[2], since the cancerous tissues of pancreatic origin were shown to contain from 2 to 4 times as much NANA as the normal control. The cause of this sialic acid increase and its functional significance in malignancy have yet to be determined. Studies of NANA concentration and of the distribution of the various macromolecular species containing NANA in subcellular fractions should help to elucidate this problem[10].

Zusammenfassung. Die Sialinsäure welche im menschlichen Krebsgewebe vorhanden ist, wurde isoliert, charakterisiert und gemessen. Im krebsartigen und normalen Gewebe wurde nur N-Acetylneuraminsäure gefunden. Die Sialinsäure im Pankreas-Adenokarzinom war ums 4fache grösser als in normalen Kontrollgeweben.

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The Effect of Surgical Trauma on Rat Secretory Neurons

Sufficient evidence elucidating the characteristic morphophysiological changes in the mammalian neurosecretory system in response to traumatic stress has now accumulated in the literature. The stressful procedures employed to induce such responses are extremely varied and range from pin pricks on rats' tails[1] to severe thermal burns on the dorsal skin of guinea pigs[2]. However, the effects of planned surgery – the commonest clinical event – on the secretory system have largely escaped attention, despite the fact that the complex regulation of post-operative fluid dynamics is almost entirely governed by the secretory axis. Consequently, the effects of surgical stress were investigated and the present report describes the structural and secretory changes in the rodent neurosecretory system following an operative trauma of moderate intensity. The morphological effects of a more severe surgical stress (spinal transection) were reported earlier[3,4].

Materials and methods. 20 male Wistar rats (250–300 g) on water ad libitum were used. Of these, 16 animals were subjected to a 3″ linear incision on their tails (the incision being carried through the skin and subcutaneous tissue upto the caudal vertebrae) under i.p. Nembutal anaesthesia (6 mg/10 g wt.) and were killed under chloroform at 8 h intervals in groups of 2. Two each of the 4 control rats were sacrificed under chloroform at 0 and 16 h respectively following initial anaesthetic doses of Nembutal.

Half the total number of operated and control animals from each group were used for cytological and cytometric studies. For this purpose, paraffin sections of gluteraldehyde-perfused material (2.5% gluteraldehyde solution buffered to pH 7.3 with 0.067 *M* cacodylate, containing 0.9% sodium chloride) of brains and pituitary glands were stained with 1% cresyl fast violet acetate. Cytometry was carried out on the supraoptic (SON) and paraventricular (PVN) nuclei with a micrometer eye-piece and the data were processed and analyzed in a computer.

The remaining animals were used for the study of neurosecretory substance (NSS). Alternate paraffin sections of formalin-fixed material were stained with chrome-alumhaematoxylin phloxin (CAHP)[5] and per-

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