pellet, which under the electron microscope appeared as an amorphous mass. However, there was a 7.2- to 14-fold increase of specific activity in the extract of the nerve-ending membranes (Table 1). In myelin, the control pellet and the extract had a much smaller uptake of dimethyl *d*-tubocurarine-C^{14}.

These results suggested that the binding capacity of the nerve-ending membranes was in some of the chemical constituents extracted with the organic solvent used. The material extracted with chloroform and methanol and a blank of 0.32*M* sucrose were washed with five volumes of distilled water. Only 5 percent of the radioactivity was recovered in the upper, watery phase of the brain extract; 99.8 percent was found in the blank. This led us to think that the gangliosides were not involved in the binding of the cholinergic blocking agent. To separate the lipids and proteolipids we passed the extract through a Sephadex G-25 column equilibrated with a mixture of chloroform, methanol, and water (60:30:4.5), which retains practically all of the nonlipid contaminants (*7*). In a control experiment, the column absorbed 98 percent of the free dimethyl *d*-tubocurarine-C^{14} dissolved in a mixture of chloroform and methanol (2 : 1) or in an extract of 0.32*M* sucrose alone. On the contrary, most of the radioactive material of the extract of nerve-ending membranes passed through the Sephadex G-25 and was recovered in the effluent. This finding differs from that recently observed by Proulx (*8*) for the uptake of γ-aminobutyric acid in brain homogenate. He observed a retention of the aminoacid by the Sephadex G-25 and postulated a rather labile type of binding. Our results may be interpreted as indicating a firmer type of binding for dimethyl *d*-tubocurarine-C^{14}. To determine whether this compound binds to the lipids or the proteolipids, we studied the effluent by thin-layer chromatography on silica gel with a mixture of chloroform, methanol, and ammonia (14:6:1) (*9*) or of chloroform, methanol, acetic acid, and water (25:15:4:2) (*10*). We found that with both methods practically all of the radioactive material remained together with the proteolipids at the point of origin.

These experiments suggest that the capacity of the junctional complex to bind dimethyl *d*-tubocurarine-C^{14} is probably due to a protein which has the solubility properties of the proteolipids. This receptor protein may be a portion of the proteolipid protein of the nerve-ending membrane, which in itself is a small proportion of the total protein (*11*). The fact that myelin has a high content of proteolipids but a very small binding capacity for dimethyl *d*-tubocurarine-C^{14} is also in line with this interpretation and indicates that the receptor protein may be a special kind of proteolipid present in some portion of the nerve-ending membrane. The results of the treatment with triton X-100 (*3, 5*) mentioned in the introduction suggest that such a proteolipid may be localized in the junctional complex of the synapse (*12*).

EDUARDO DE ROBERTIS
SARA FISZER
EDUARDO F. SOTO
Instituto de Anatomía General y Embriología, Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

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Distribution of Chromatids at Mitosis

Abstract. *The distribution of labeled chromatids at the second mitosis after labeling with tritiated thymidine is random in both* Vicia faba *(the broad bean) and* Potorous tridactylis *(the rat kangaroo). This finding is contrary to that predicted by the hypothesis that chromatids containing "grandparent" polynucleotide templates segregate from those containing "parent" templates.*

Lark, Consigli, and Minocha (*1*) have recently suggested that the segregation of chromatids at mitosis is nonrandom, that is, that all chromatids containing subunits synthesized in the previous generation segregate together. Thus, at the second mitosis after labeling with tritiated thymidine, all labeled chromatids would proceed to one pole, and all unlabeled chromatids would go to the other. This possibility, suggested by experiments on bacteria (*2*), was apparently confirmed by the frequency distribution of grain counts over nuclei at various times in primary cultures of mouse embryos, and in cultures of hamster cells. Lark and his co-workers recognized that the formation of sister-strand exchanges between labeled and unlabeled chromatids would tend to disrupt the pattern. The hypothesis has since been extended to *Vicia faba* (the broad bean) and *Triticum boeoticum* (wheat) (*3, 4*). The phenomenon might, therefore, be general.

The suggestion of complete nonrandom segregation of chromatids is contrary to the usual assumptions that have been made (for example, *5*), and is contrary to our previous qualitative observations. To obtain quantitative results from experiments that avoid the

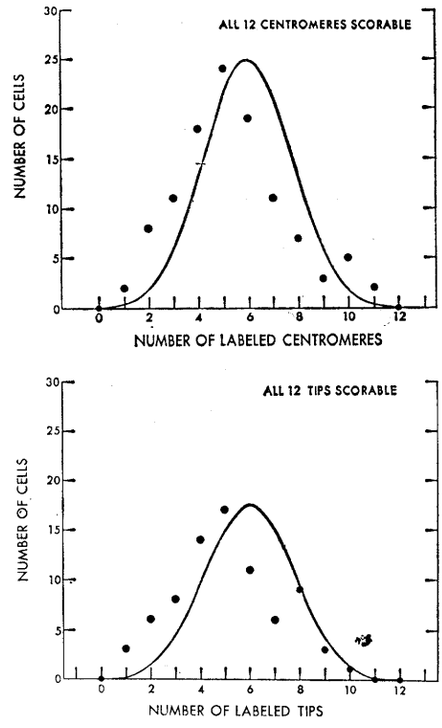

Fig. 1. Frequency distribution at the third mitosis after labeling of *V. faba* root tips. The line is the binomial expected from a random segregation of all 12 elements if the probability of being labeled is 0.5. (Top) The frequency of cells having 0,1,2 . . . 12 labeled centromeres. (Bottom) The frequency of cells having 0,1,2 . . . 12 labeled chromosome tips.

difficulties presented by (i) sister-strand exchange, and (ii) variable cell cycle durations, we have examined the proportion of labeled and unlabeled centromeres in cells dividing for the third time after being labeled with tritiated thymidine. Metaphases of the third division were chosen because it is at this stage that one can see the results of the segregation of centromeres that occurred at the second mitosis.

Vicia faba beans were grown at 20° ± 1°C, were fixed, and slides made according to the technique described by Wolff (*6*). Seedlings with lateral roots were placed in $3.6 \times 10^{-3}M$ 5-amino uracil for 12 hours to synchronize the cells. Two hours after removal from 5-amino uracil, the roots were placed in tritiated thymidine (1μc/ml; specific activity, 1.9 c/mmole) for 2 hours, then in water for 45 hours. They were then placed in an aqueous solution of $2 \times 10^{-3}M$ colchicine for 11 hours to arrest cells in the third metaphase after labeling. At the end of this time, the cells were fixed, and slides were made. Autoradiograms were made by dipping the slides in Ilford L-4 liquid emulsion and storing them for 51 days.

Third mitoses were distinguished from second mitoses by the presence of iso-unlabeled regions (regions in which there are no grains over either chromatid). This was possible because the high frequency of sister-strand exchanges that is observed in the second division (*7*) would result in iso-unlabeled regions at the third mitosis, regardless of the segregation pattern.

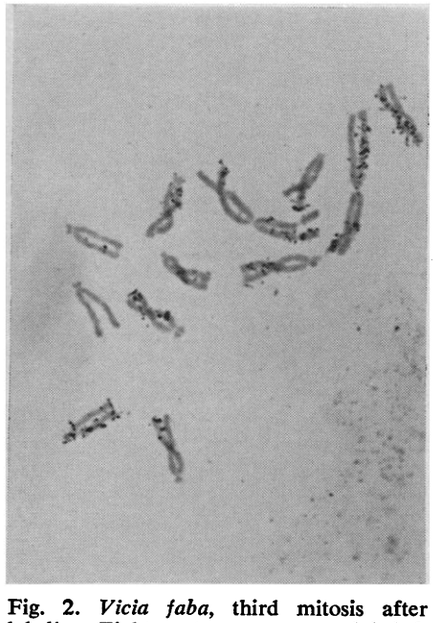

Fig. 2. *Vicia faba*, third mitosis after labeling. Eight centromeres are unlabeled, and four are labeled.

Centromeres were considered suitable for scoring only if they were not overlapped by another chromosome and if no other chromatid lay within one chromatid's width (about 1 μ) of the centromere. A centromere having two or more grains within one chromatid's width along the chromosome (in either direction) was considered labeled; a centromere having only one or no grains was considered unlabeled. In some cells not all 12 centromeres were suitable for scoring. Figure 1 (top) shows the frequency distribution of labeled centromeres in cells having 12 scorable centromeres. The line represents the theoretical binomial distribution expected if centromeres segregate at random. Similar results were obtained in cells with 11 scorable centromeres, and in those with ten (*8*); cells with fewer suitable centromeres were excluded. A typical cell is shown in Fig. 2. A nonrandom segregation would result in a bimodal curve with peaks at the numbers of centromeres that segregate together; in the particular case in which all labeled centromeres segregated together, the peaks would be at 0 and 12 labeled.

Although the distribution of cells is binomial, the mean is evidently lower than the theoretical mean of six labeled centromeres. This shift in mean results from characteristics of the autoradiographic technique itself. Some labeled centromeres will fail to have two or more grains over them because they represent the zero- and one-grain classes of the Poisson distribution. Still others will fail to have two or more grains because of strictly technical artifacts (*9*). Both factors would lead to an underestimate of the numbers of centromeres labeled and thus shift the distribution towards more unlabeled centromeres. Some of the shift could also be a result of the presence of a few cells in the fourth mitosis after labeling.

That autoradiographic artifacts are responsible for the reduced mean is supported by data on the chromosome tips. Chromosome tips are expected to show more or less random distribution even if centromeres do not, since sister-strand exchanges would disrupt any pattern determined by the centromeres. Nevertheless, the data in Fig. 1 (bottom) show that distributions obtained for tips were similar to those obtained for centromeres. To make the distribution of tips comparable to that for centromeres, we restricted our scoring to the tips of the long arms and required more than one grain in a span of two chromatid widths from the end. Again, the distribution is shifted towards fewer labeled tips, but it is the binomial distribution expected for random segregation. In *V. faba*, it seems clear, therefore, that chromatids of chromosomes are distributed at random to daughter cells.

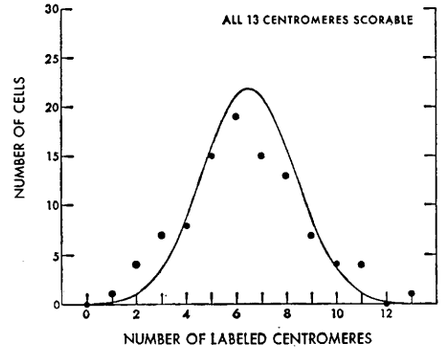

Fig. 3. The frequency of cells having 0,1,2 . . . 13 labeled centromeres at the third mitosis after labeling in *P. tridactylis*. The line is the binomial expected for a random segregation of all 13 centromeres if the probability of being labeled is 0.5.

Similar results have been obtained with asynchronous cultures of an aneuploid cell line of *Potorous tridactylis*, the rat kangaroo (Fig. 3). The cells were fixed after a 4-hour treatment with $8 \times 10^{-5}M$ colchicine at 49 to 55 hours after labeling (1μc/ml; specific activity, 1.9 c/mmole; 20 min-

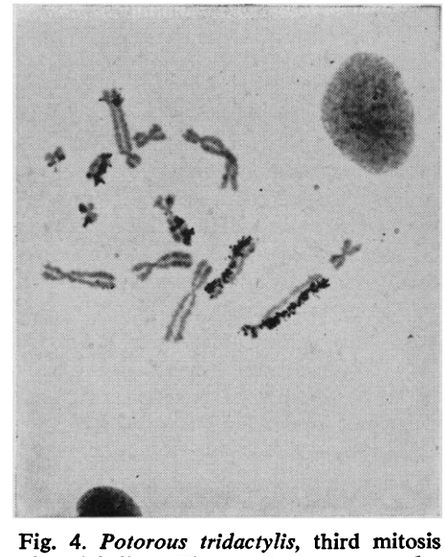

Fig. 4. *Potorous tridactylis*, third mitosis after labeling. Five centromeres are labeled, and eight are unlabeled.

ute pulse). We scored only cells in which all 13 centromeres were scorable, for example, Fig. 4. Once again it is evident that the distribution of chromatids is random: all labeled chromatids do not inevitably proceed to one pole. In addition to these quantitative results on the broad bean and the rat kangaroo, qualitative results consistent with random segregation have been obtained by others [for example, in human leukocytes (*5*), Chinese hamster cells (*10*), and onion root tips (*11*)]. We believe, therefore, that random segregation is the general rule.

The idea that chromatids might segregate nonrandomly is based upon (i) unequal labeling of daughter nuclei and (ii) the frequency distribution of grains over cells. Unequal labeling of daughter nuclei could arise in a variety of ways, including random as well as nonrandom segregation of chromatids. For instance, in *V. faba* with 12 centromeres segregating randomly at the second mitosis, less than 23 percent of the sister chromatid sets at anaphase would be equally labeled (*12*). Under the same assumptions (*12*), the weighted mean ratio of lightly labeled to heavily labeled sister chromatid sets at anaphase will be 0.67.

The frequency distribution of grains that led Lark to suggest nonrandom segregation of chromatids in *V. faba* (*4*) were obtained at the time when third mitoses after labeling were expected (although the binomial calculation was for second mitoses). Because of asynchrony and the difficulty of determining which mitosis the cells are in, the population observed by Lark (*4*) must have been a mixture of cells in different mitoses after labeling. Even in our experiments in which we synchronized the roots before labeling them, we found that, at the time when third mitoses were expected, second, third, and even first mitoses were present. Furthermore, we found some mitoses that had only about one eighth of the chromatin labeled and so were probably fourth mitoses (although we could not exclude them from our scoring as they could have been third mitoses in which most unlabeled chromatids went to one pole). Thus, even though the cells were labeled at one time, subsequent asynchrony must have distorted the frequency distributions of grain counts in Lark's experiments. We believe that it is this factor which led to the conclusion that chromatids of animals and plants segregate nonrandomly. Our results demonstrate that in the case of *V. faba* and *P. tridactylis* chromatids do segregate randomly at mitosis.

JOHN A. HEDDLE
SHELDON WOLFF*
D. WHISSELL†
JAMES E. CLEAVER
Laboratory of Radiobiology, University of California Medical Center, San Francisco

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13. We thank Miss D. Judy Bodycote for her assistance. Work performed under the auspices of the AEC and of PHS grant 5 T1 GM-367-07.

* Joint appointment in Department of Anatomy, University of California Medical Center, San Francisco.
† Department of Genetics, University of California, Berkeley.

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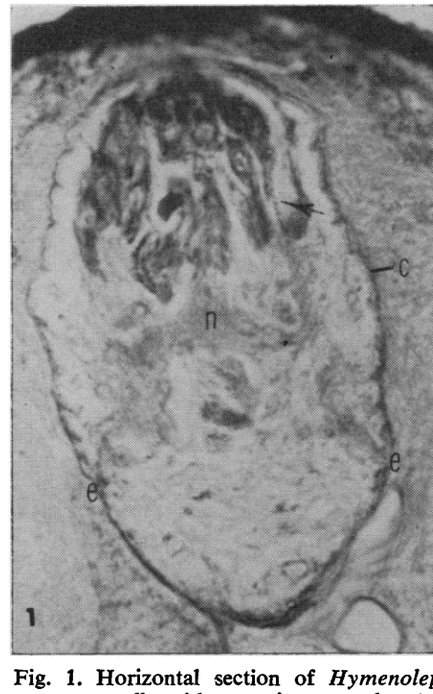
Neurosecretory Cells in a Cestode, Hymenolepis diminuta

Abstract. *A group of nerve cells in the rostellum become progressively more fuchsinophilic during the first 16 days of development; they then release their secretion into their axons at about the time that the first proglottid is released.*

Neurosecretory cells are well known from the free-living flatworms (*1*), and their presence in cestodes has been suggested on the basis of evidence from the electron miscroscope (*2*). Using the paraldehyde-fuchsin technique of Cameron and Steele (*3*), we have demonstrated neurosecretory cells in the scolex of the tapeworm *Hymenolepis diminuta*. The tapeworms were reared by feeding cysticercoids recovered from adult flour beetles (*Tribolium confu-*



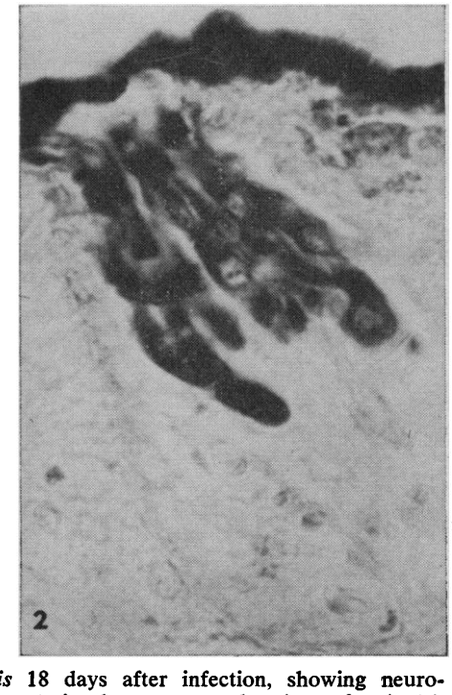


Fig. 1. Horizontal section of *Hymenolepis* 18 days after infection, showing neurosecretory cells with secretion granules (arrow) in the axons, and points of exit (*e*) of nerve tract (*n*) through rostellar capsule (*c*) (× 11,000). Fig. 2. Similar section showing neurosecretory cells in a worm 7 days after infection. Note swollen appearance of cells with abundant secretion (× 1400).