out that the DL compounds **5**, **8** were prepared from DL-aminobutyric acids. By analogy with the compounds **2**–**2 D** and **3**–**3 D** we expect that the biologically active components of the racemates were only their L-forms.

All the compounds listed in Table I were subjected to additional topical or injection bioassays on a Pentatomid bug *Graphosoma* and on pupae of *Tenebrio*. No JH activity was recorded here and also in other orientation tests performed on *Gryllus bimaculatus*, *Dermestes vulpinus*, *Drosophila melanogaster*, etc. Evidently, all these compounds are highly specific, acting only on the insects of the family

Pyrrhocoridae, similarly like the JH analogues of juvabione type². Contrary to the optical isomers of juvabione⁸, which all retain the JH activity², the compounds we have described are active only when containing the natural L-amino acids. This phenomenon can perhaps be explained by a more rigid structure of the molecules containing amidic bonds.

In order to obtain more information on the specificity and structure-activity relationships of these hormone analogues, we have prepared series of compounds in which other parts of the molecule have systematically been altered. The results will be published later.

Cell Cycle Analysis and Stimulation of Rat Kangaroo Cells (PtK₂) after Pulse Labeling

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The phases of the cell cycle of *Potorous tridactylus* (PtK₂) cells were determined *in vitro* by analysis of labeled mitoses for 37.1/2 hours after a tritiated thymidine pulse. The mean duration of DNA synthesis (*t*_S) was 8 h. The mean duration of the gap phase before appearance of labeled mitoses was 5 h. Whereas the duration of the cell cycle (*t*_C) based on analysis of labeled mitoses was 30 h, the doubling time (*t*_D) derived from cell counts in the same cultures was only about 23 h. The analysis of the indices of labeled nuclei and mitoses suggests a stimulation of cells at the time of pulse labeling, which was maximal after beginning of the gap phase before DNA-synthesis, and possibly caused the observed difference between *t*_C and *t*_D.

The cell cycle time (*t*_C) of permanent cell lines established from kidneys of the rat kangaroo, *Potorous tridactylus* has been found to be 28 to 32 h¹. No data on the phases of the cell cycle of *Potorous tridactylus* cells have been published². Although the duration of DNA-synthesis (*t*_S) of mammalian cells was often found to be close to 8 h³, significant variations have been described^{2–4}. Since cells of *Potorous tridactylus* are ideally suited for chromosomal studies^{5–7}, we determined the cell

cycle parameters of PtK₂ cells in non-synchronized monolayer cultures by analysis of labeled mitoses. The results were related to the doubling time (*t*_D) of the same cultures.

Cells (2.5 × 10⁴ per ml) were suspended in EAGLE's minimum essential medium⁸ containing 10 percent fetal bovine serum, and seeded on cover glasses in 6-cm Falcon Petri dishes. After 3 days of incubation in an atmosphere of humidified air containing 5 percent CO₂, this medium was replaced for 10 to 17 min with medium containing 7 × 10^{–7} M tritiated thymidine

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(^3H -TdR) (Amersham Searle; specific activity 20.2 or 29 mCi/mM) for "pulse" labeling. The cells were subsequently washed with medium without thymidine, and then incubated in medium containing 7×10^{-6} M or 2.1×10^{-5} M of "cold" thymidine. Cultures were sampled at 1 to 1-1/2 h intervals for 37-1/2 h, fixed in methanol-acetic acid (3:1), air-dried, and overlaid with Kodak AR-10 stripping film according to PELC⁹ and SCHMID¹⁰. The films were exposed for 7 days and developed with Kodak D-19 solution. The cells were Giemsa-stained¹⁰ through the film. Labeling indices of mitoses and total nuclei were determined by counting 100 mitoses and at least 1000 nuclei per culture, respectively. The growth rates of the cultures were determined by measuring the areas scanned for counting of nuclei.

Fig. 1. Growth of PtK₂ cells; least square fit of average numbers of nuclei counted for several time intervals in two experiments shows a value of 23 h for t_D .

Depending on the technique of analysis, different values were obtained for the cell cycle time (t_C) and t_D , respectively. t_D derived from cell counts was about 23 h (Fig. 1). The analysis of the labeled mitoses curve gave about 30 h for t_C , a value which agrees with the results of WALEN¹ (Fig. 2, curve a). t_S was about 8 h. The first labeled mitoses appeared after a gap phase (minimum t_{G_2}) of approximately 3 h after the beginning of the pulse. The mitotic index at the beginning of the chase was between 2 and 4%, indicating that the average duration of mitosis was approximately 1 h (Fig. 2 upper part). The time from the end of DNA-synthesis (S) to the beginning of the next S-period was estimated by determining the increase of the labeling index in the presence of ^3H -TdR in the culture medium for up to 25 h. Asynchronous cultures of PtK₂-cells were pulsed for

Fig. 2. Upper part: Mitotic index of PtK₂ cells; the two curves indicate the range of 2 standard deviations calculated for 3 experiments. Lower part: Labeling indexes of mitoses (black symbols, curve a) and total nuclei (open symbols, curve b) obtained in 3 experiments. As measured at the 50 percent level of curve a, t_C was 30 h; t_S was 8 h; $t_M + t_{G_1}$ was 19 h; the duration of $G_2 + 1/2 M$ 5 h; and the minimal duration of G_2 was 3 h.

various time intervals under conditions similar to those described for the pulse-chase experiments, except that the chase was omitted. The cultures were then fixed, and autoradiographs were prepared as described above. Approximately 22% of all nuclei were instantly labeled, and within the following 21 h the labeling index (least square regression analysis) rose to 100% (Fig. 3). This result is considered to be in fair agreement with the value of 22 h for $t_C - t_S$ obtained from the analysis of labeled mitoses. The curve for the labeling index as a function of time after pulse labeling shows a significant increase by 20% ($p < 0.0025$ by t -test) towards the end of the first peak of labeled mitoses, which lasts for approximately 9 h, and reaches a maximum 18 h after the pulse (Fig. 2, curve b). This agrees with the type of curve expected after pulse labeling in an asynchronous population of cells in the exponential phase of proliferation. However, a significant in-

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Fig. 3. Labeling index of PtK₂ cells as a function of time of exposure to ³H-TdR; two experiments. Rate of ³H-TdR incorporation into PtK₂-cells.

crease by 60 to 100% ($p < 0.05$) in the mitotic index, mainly due to unlabeled mitoses, paralleled the increase in the labeling index, lasted for approximately 14 h, and showed a maximum ($p = 0.025$) 21 h after the pulse (Fig. 2).

When the increase of mitoses is related to the time elapsed after the pulse, the phase of the generation cycle during which stimulation occurred can be determined (Fig. 4). Stimulation occurred in G₁ and was maximal after the beginning of G₁. Variations of t_{G_1} have been described as responsible for changes in the generation cycle of rat and mouse cells¹¹. Recently a certain fraction of fetal bovine serum has been isolated which effectively stimulated rat cells prior to S and was essential for completion of the cycle¹². Correspondingly, environmental changes occurring simultaneously with the radioactive labeling of PtK₂-cells may have resulted in

the stimulation of a subgroup of G₁-cells. This may account for the observed difference between t_D and t_C . It is, however, important to note that the analysis of labeled mitoses curves gives precise results only when the technique is applied to an asynchronous cell population. Since, on the basis of the curve for the mitotic index (Fig. 2), a partial synchronization of the PtK₂-population is assumed, the cell cycle parameters described may deviate to some extent from the real values for a strictly asynchronous system. The possibility that certain subpopulations of asynchronous cell systems may be stimulated by environmental changes must be considered in experiments in which cell cycle parameters have been determined by combining analyses of labeled mitoses with total cell counts¹³.

Fig. 4. Generation cycle of PtK₂ cells (30 h) and its phases, see text for explanation of symbols. Circular sections outside the generation cycle indicate the observed increases of labeled nuclei and non-labeled mitoses relative to the time of the pulse. Increase of non-labeled mitoses was maximal towards the end, and was stimulated during G₁; increase of total labeled nuclei was due to selective labeling of S-phase cells during the ³H-TdR pulse.

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