Ausdruck. Überall im Bindegewebe juveniler Weinbergschnecken finden sich markierte Zellkerne in unregelmässiger Verteilung (Figur 2), und zwar 6 h bis 8 Tage nach der Injektion der «Tracers». Die adulten Vt besassen im April kurz nach der Winterruhe im Bindegewebe nur sehr wenige markierte Zellkerne, deren Zahl nach 36tägiger Inkubation im August zwar grösser war, aber nicht die Teilungsaktivität des juvenilen Bindegewebes erreichte. Im Bindegewebe um die Schlundringganglien, im Augenfühler und unter der Epidermis wiesen weder Muskel- und Blasenzellen noch Körnchen- und Pigmentzellen markierte Zellkerne auf. Bei der Inkorporation des «Tracers» in Bindegewebeszellkerne muss es sich also um Kerne noch nicht ausdifferenzierter Fibrozyten handeln, oder um Fibroblasten, die als embryonale Bindegewebeszellen auch noch im Bindegewebe adulter Schnekken vorkommen und sowohl im Frühjahr als auch im Sommer Teilungen durchführen. Auffällig waren die zahlreichen Markierungen der Kerne von Blutzellen bei juvenilen Vt (Figur 2), was in stark verminderter Zahl auch noch bei adulten Tieren festzustellen war. Ausserdem zeigte sich, wiederum bei juvenilen Tieren vermehrt, dass im Zellverband, der das Lumen der Gefässe auskleidet, stets markierte Kerne vorhanden waren, die sich teilweise mit ihren Zellen in das Gefässlumen vorwölbten. Es besteht daher die Möglichkeit, dass ein Teil der Blutzellen von *Helix pomatia* durch Mitosen bestimmter Zellen in der Gefässwand entstehen. Zum Abschluss soll noch erwähnt werden, dass in der einschichtigen Epidermis juveniler und adulter Weinbergschnecken stets markierte Zellkerne anzutreffen waren. Es scheint sich dabei um Teilungen epidermaler Ersatzzellen zu handeln, die in unregelmässiger Verteilung zwischen den hochprismatischen Epithelzellen vorkommen. Das Bindegewebe unter der Epidermis enthielt im Vergleich zum Bindegewebe um die Schlundringganglien – auch bei juvenilen Vt – nur eine geringe Zahl an markierten Zellkernen. Es kann daher vermutet werden, dass hierin ein Grund für die bei Gastropoden bekannte mangelnde Regenerationsfähigkeit der Haut zum Ausdruck kommt.

Zusammenfassend kann man feststellen, dass in den bisher untersuchten Organen und Geweben von juvenilen und adulten *Helix pomatia* stets Zellteilungen vorkommen. Eine Vermehrung der Nervenzellen durch Teilungen ist in den zentralen Ganglien wahrscheinlich früh in der Ontogenese abgeschlossen[2], eine Grössenzunahme der Nervenzellen in der letzten Wachstumsphase juveniler zweijähriger Tiere kann vermutlich nur noch durch Polyploidisierung erfolgen[3]. Zur bekannten Grössenzunahme der Ganglien[2] tragen wahrscheinlich auch die zahlreichen Teilungen im Gliagewebe bei, die auch bei adulten Tieren noch auftreten können und wahrscheinlich einen Ersatz degenerierender Gliazellen darstellen. Ähnliches gilt für die Teilungsaktivität im Bindegewebe und der Epidermis erwachsener Schnecken. Die Mitosen in den untersuchten Organen juveniler Tiere stehen überwiegend im Dienst der Körper- und Organvolumenzunahme, während die bei adulten Weinbergschnecken in geringer und unterschiedlicher Zahl festgestellten markierten Zellkerne Teilungen als Ersatz für degeneriertes Zellmaterial darstellen dürften.

Summary. It has been demonstrated by injections of H^3-Thymidine into adult and juvenile snails (*Helix pomatia*) that the mitotic activity in the central nervous system, the connective tissue and epidermis was generally higher in juvenile animals than in adult ones. Data are given for the amount of triated thymidine labelled nuclei in the tissues, which are investigated.

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Inhibition of Novikoff Ascites Cell Transplantation by a Polysaccharide Component in Bull Seminal Plasma

A neutral polysaccharide is present in bull seminal plasma which chemically reacts with and partially degradates tRNA, rRNA, viral RNA and polyuridylic acid[1-3]. Native and denatured DNA are resistant to the polysaccharide. Transfer RNA and rRNA methylation and tRNA aminoacylation were totally inhibited by a 1:3000 dilution of a partially purified deproteinized extract of the seminal plasma. Dilutions of the inhibitor solution as low as 1:500,000 were observed to exert 25% inhibition of enzymatic reactions which utilize tRNA or rRNA as a substrate.

In the present communication, data are presented to show that a similar polysaccharide extract prepared from bull seminal plasma totally inhibits the transplantation of the rapidly growing Novikoff ascites hepatoma.

Materials and methods. The Novikoff ascites hepatoma was transplanted intraperitoneally every 7th day (10 ml of ascites fluid/kg). Approximately 50 ml fluid accumulated in the abdominal cavity of each rat, with death resulting in 9–10 days. Whole bull semen collected over liquid nitrogen was kindly supplied by The Eastern Artificial Insemination Corporation, Ithaca, New York. No antibiotics or preservatives were added to the final pooled sample. Seminal plasma was separated from the sperm cells by centrifugation at $850 \times g$. Three test solutions were used for injecting the animals. For solution A, a 1:100 dilution of seminal plasma in distilled water was dialyzed against 4 changes of 4 l of water for 24 h at 4 °C. For solution B, solid trichloroacetic acid was added to a 1:80 dilution of seminal plasma until the plasma was 7% with respect to acid. After 30 min at 4 °C, the deproteinized solution was centrifuged and the supernatant neutralized with concentrated NaOH (final pH 7.4). A final dilution of 1:100 was then dialyzed in the same manner as solution A. For solution C, a 1:80 dilution of deproteinized seminal plasma was

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incubated in the presence of $2\times10^{-2}M$ $NaIO_4$, $0.005M$ phosphate buffer pH 6.8 and 2 mg of tetrasodium EDTA in a volume of 9 ml for 1 h at 37 °C. Following this, 1 ml of $0.025M$ glucose was added and the mixture reincubated at 37 °C for 1 h to destroy the excess periodate. This solution was then dialyzed as solutions A and B. In some experiments the test solutions were preincubated with pronase and trypsin to insure the removal of trace amounts of protein. The proteases were subsequently destroyed by heating at 100 °C, a procedure which has no effect on the integrity of the polysaccharide. 1 ml of all of the test solutions was then incubated with 1 ml of ascites fluid at 37 °C for 30 min. 1 ml portions of these mixtures were then injected into the various groups of animals. The animals not receiving seminal plasma were injected with ascites fluid preincubated with saline.

Results and discussion. The Table shows that transplantation of the Novikoff ascites hepatoma was totally inhibited if the ascites cells were preincubated with a deproteinized and nucleic acid-free, dialyzed extract of seminal plasma. In these experiments, a 1:200 dilution of seminal plasma was employed as described in the section on methods. In further studies, it was also possible to completely inhibit the transplantation of the tumor by a 1:500 dilution of the seminal plasma in ascites fluid. A 1:1000 dilution resulted in a slower growing hepatoma in which the survival time of the rats (3 subjects) increased from 9–10 days to 33–35 days. Rats administered a 1:2500 dilution of the seminal plasma survived for 15–20 days. A 1:5000 dilution had no effect on the viability of the tumor or the survival time of the rats. It may be inferred from this data, and the fact that periodate oxidation destroyed the inhibitory effect of the seminal plasma, that the antitransplantation factor is a polysaccharide(s). The polysaccharide may function in vivo by penetrating the ascites cells and reacting chemically with the cellular RNA as demonstrated in vitro[2,3]. Another possibility is that the polysaccharide may bind to the ascites cell membranes to prevent multiplication of the cells in the host animals. Rats injected with ascites fluid preincubated with untreated seminal plasma survived 2.5 times longer (20 to 27 days) than rats injected with just ascites fluid (9–10 days). However, on autopsy, there was no evidence of any tumorous ascites cells in the animals injected with ascites cells plus seminal plasma. The demise of these animals, and of animals treated with untreated seminal plasma alone (20–25 days) may have been due to the toxic effects of substances which were excluded by precipitation with trichloroacetic acid.

Survival time of rats injected with Novikoff ascites cells preincubated with various preparations of bull semen seminal plasma

Animal No.	Addition to ascites fluid[a]	Presence of ascites hepatoma cells[b]	Days of survival[c]
1	None	+	9
2	None	+	9
3	None	+	10
4	Seminal plasma	—	20
5	Seminal plasma	—	23
6	Seminal plasma	—	27
7	TCA treated seminal plasma	—	>42
8	TCA treated seminal plasma	—	>42
9	TCA treated seminal plasma	—	>42
10	Periodate and TCA treated seminal plasma	+	8
11	Periodate and TCA treated seminal plasma	+	12
12	Periodate and TCA treated seminal plasma	+	12

[a] TCA is the abbreviation for trichloroacetic acid. [b] (+) signifies the presence of ascites hepatoma cells. (—) signifies the absence of the cancer cells. [c] The experiment was of 42 days' duration were the results were communicated.

Once the transplantation of the ascites cells was completed, it was not possible to inhibit the growth of the tumor, or to increase the survival time of the rats with injections of seminal plasma extracts. Various concentrations of the extract at different times after the transplantation were tried without success. In these cases, the polysaccharide preparation may have been diluted out in the whole animal, or there was a lack of sufficient contact between the ascites cells and the inhibitory substance. Also, the animals may have a metabolic mechanism to inactivate the polysaccharide.

It would be of interest to isolate and purify the antitransplantation factor and test its potential in more concentrated amounts as an antitumor agent on different types of tumors of both chemical and viral etiology. A number of neutral polysaccharides have been described which possess antitumor properties[4].

Resumen. Un extracto desproteinizado preparado a partir de plasma seminal de toro, inhibió completamente la transplantación del hepatoma ascítico de Novikoff de rápido crecimiento. Se sugiere a partir de los experimentos realizados que el factor activo antitransplantación es un polisacárido.

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Neuron in the Gracile Nucleus with Myelinated Axon Hillock

The initial axon segment and the axon hillock is the part of the multipolar nerve cell, where the action potential is thought to originate[1]. It has been recognized and described ultrastructurally[2]. The studies have shown the same basic structural features: After the transition of the cell body to the axon proper known as the axon hillock the unmyelinated axon known as the initial axon segment starts. The last part of the hillock and the initial axon segment are characterized by an irregular undercoating on the cytoplasmic side. The axon hillock but usually not the initial axon segment is contacted by boutons.