(Figur 1). Ein erstes Maximum liegt am 3. Tag, danach wird das Ausmass der Hemmung deutlich geringer, um vom 5. Tag an stufenweise wieder zuzunehmen, bis zwischen dem 12. und 13. Tag ein kompletter Ausfall der Makrophagenmigration deutlich wird (Figur 2).

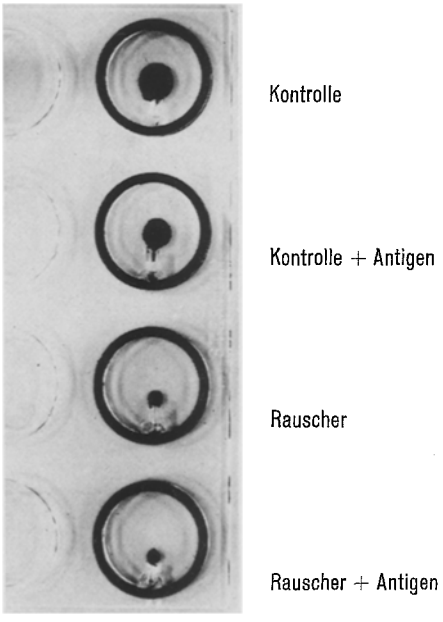

Fig. 1. Makrophagen Migrations-Hemmtest in verschiedenen Versuchsansätzen. Im Gegensatz zu den Kontrollen tritt bei den mit RAUSCHER-Virus infizierten Tieren eine stärkere Hemmung auf.

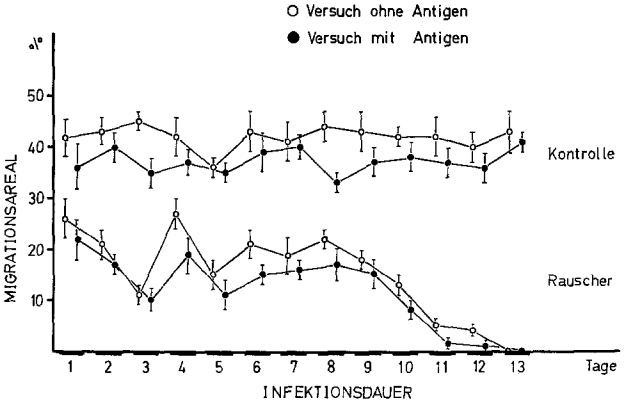

Fig. 2. Schematische Darstellung des Migrationstestes während der Beobachtungszeit.

Die vorliegenden Beobachtungen zeigen, dass während der Frühphase der RAUSCHER-Leukämie eine verminderte Wanderung von Makrophagen stattfindet, die einen charakteristischen Verlauf zeigt. Der Kurvenverlauf bis zum 5. Tag lässt sich nicht einheitlich erklären. Die auffallend starke Hemmung am 3. Tag muss auf eine spezifische immunologische Antigenstimulation der T-Lymphozyten zurückgeführt werden. Am 4. Tag wird eine geringere Hemmung der Wanderung der Makrophagen deutlich. Hierfür scheinen quantitative Veränderungen der Lymphozyten der «thymus dependent area» für den Reaktionsausfall verantwortlich zu sein. Diese Interpretation deckt sich mit unseren Ergebnissen über die Proliferationsprozesse während der Frühphase der Rauscherleukämie in der Milz (KOMITOWSKI et al.[7]). In den ersten 5 Tagen nach der Infektion zeigte sich erst eine Verminderung, dann eine Repopulation der Lymphozyten der «thymus dependent area». Inwieweit der Reaktionsausfall ab dem 5. Tag auf eine spezifische immunologische Antigenstimulation der T-Lymphozyten, oder auf eine dynamische Veränderung der Makrophagen selbst, wie von anderen Autoren (CHAN et al.[11]) postuliert, zurückzuführen ist, bedarf weiterer Abklärung.

Summary. In the course of virus-induced leukemias, there are changes in cellular as well as in humoral immunity. Observations were made with the macrophage migration test during the early phase of RAUSCHER-leukemia. The characteristic phase changes found are discussed.

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Partially Coated Vacuoles — a New Type of Endocytotic Structure

Micropinocytotic vesicles bearing filamentous or spiny adornments on their limiting membrane have often been seen in cells engaged in protein-uptake, and terms such as fuzzy vesicles, coated vesicles, decorated veiscles and acanthosomes have been used to describe this type of endocytotic vesicle[1, 2]. However, to the best of our knowledge, such adornments have not hitherto been observed on the much larger pinocytotic vacuoles which form when folds or ruffles of the cell membrane (which are present as slender processes or filopodia in sectioned material) impound fluid or other extracellular material.

Such a phenomenon has now been witnessed by us in the synovial cells of a patient who had long standing multiple sclerosis, psoriasis and arthritis. Electron microscopic examination of synovial intimal cells found free in a knee joint effusion and in a biopsy of the synovial

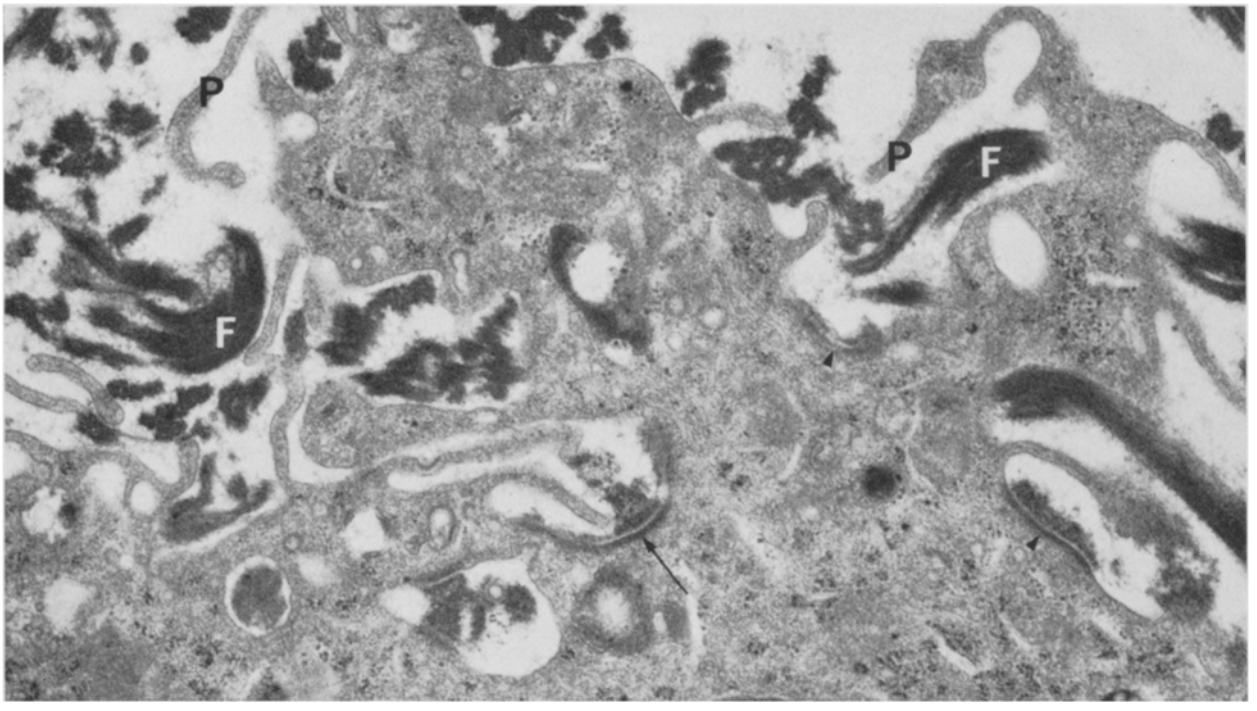

Fig. 1. Electron micrograph showing fibrin (F) in joint space and a synovial cell with numerous filopodia (P). Note dense plaques (arrowheads) one of which is cup-shaped (arrow). ×27,000.

membrane obtained with a Poly-Bickel needle showed that they were singularly well endowed with filopodia which appeared to be engaged in trapping fibrin lying in the joint space (Figure 1).

The remarkable endocytotic powers of synovial cells have been amply documented and reviewed[3]. It has been shown that they can take up not only small particulate matter such as carbon, thorotrast and colloidal gold with the aid of micropinocytotic vesicles, but also larger structures such as entire erythrocytes, cell fragments and masses of fibrin by the aid of filopodia which fuse with each other or the cell membrane to form pinocytotic vacuoles.

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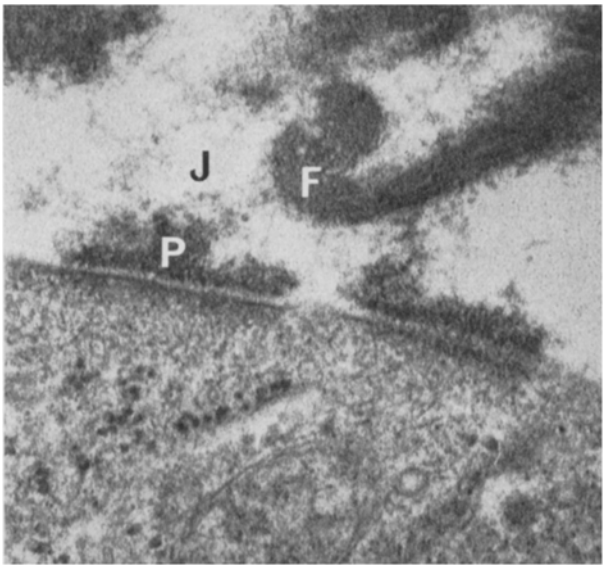

Fig. 2. Two dense plaques (P) are seen on a synovial cell. The material forming the external coat of the plaque is similar in appearance to the fibrin (F) lying in the joint space (J). ×62,000.

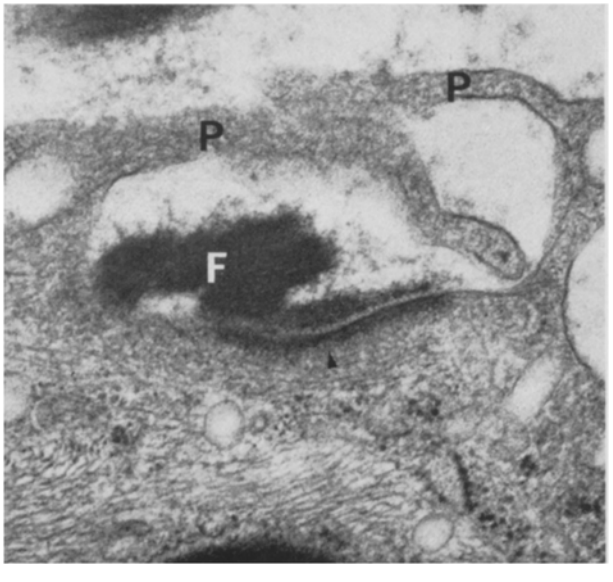

Fig. 3. Filopodia (P) encircling fibrin (F). A dense plaque is seen on the surface of the synovial cell (arrowhead). ×53,000.

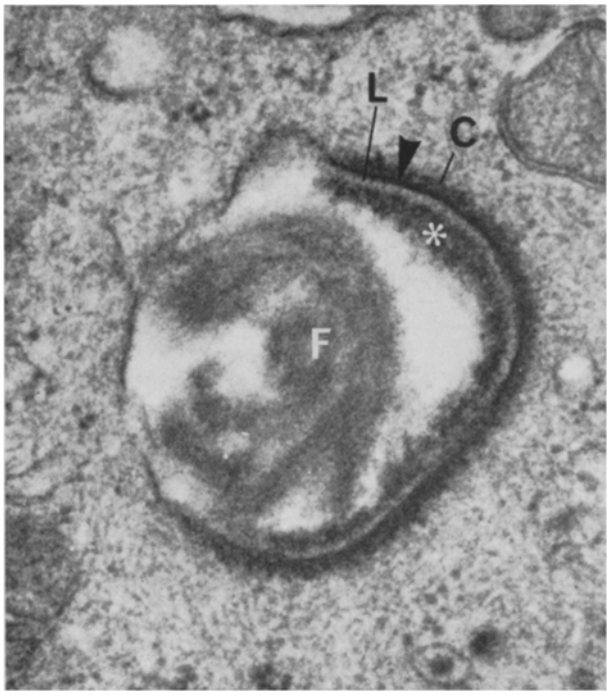

Fig. 4. A fibrin (F) containing partially coated vacuole within a synovial cell. A little more than half the circumference of the vacuole is coated. Note the dense filamentous coat (C) on the cytoplasmic surface of the vacuolar membrane (arrowhead) and the less dense (L) interval between the membrane and the dense coat (*) lining the vacuole. ×70,000.

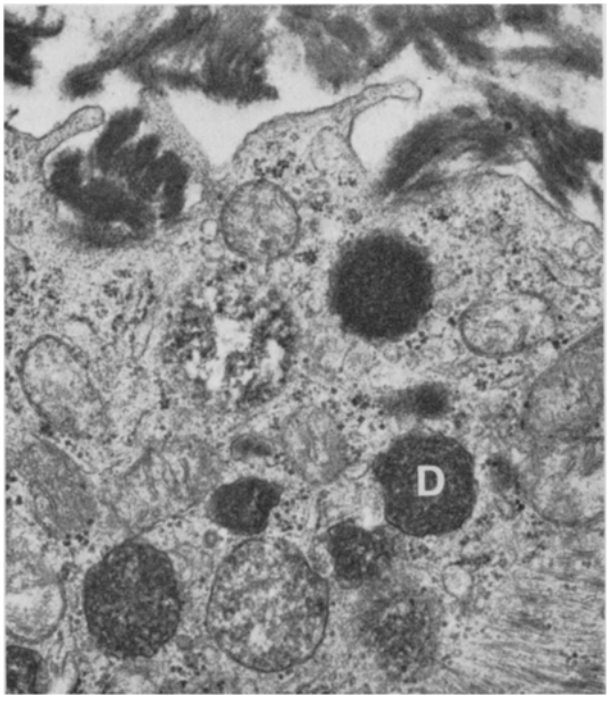

Fig. 5. Synovial cell containing numerous dense bodies (D) acceptable as phagosomes and phagolysosomes. ×28,000.

The uptake of fibrin and cell fragments by pinocytotic vacuoles has been noted in the rheumatoid synovial membrane as also the abundant phagosomes and phagolysosomes which form from such activity[3,4]. In such instances, however, the vacuoles are not coated. In the case reported in this paper, the uptake of fibrin was largely accomplished with the aid of partially coated vacuoles (up to 800 nm in diameter), that is to say vacuoles where only a portion of the circumference of the vacuole was coated and bore filamentous decorations on its cytoplasmic surface.

A detailed study of numerous synovial cells from this case permits us to depict the probable stages in the formation of these vacuoles. The first stage of this process (Figure 2) is rather similar to the formation of coated micropinocytotic vesicles, such as those found in erythroblasts taking up ferritin[5] or insect oocyte taking up protein to form yolk bodies[6,7], for at such a stage a dense plaque develops on the cell membrane.

Closer examination shows that this plaque consists of a focal acquisition of an electron dense filamentous coat, approx. 35 nm thick on the cytoplasmic aspect of the plasma membrane, and a similar coat about 50 nm thick on the outer surface of the membrane. However, the outer surface coat was invariably separated from the plasma membrane by a less dense or lucent lamina, about 20 nm thick, while the coat on the cytoplasmic surface was closely attached to the plasma membrane.

In the case of coated micropinocytotic vesicles (usually about 80 to 140 nm), such a stage is followed by an invagination of the dense plaque to form the micropinocytotic vesicle. It is interesting to note that during the formation of the partially coated vacuoles also, we saw saucer-shaped and cup-shaped plaques indicating that invagination of the plaque had commenced but in no instance did the process continue to form a fully coated vacuole, that is to say a vacuole coated along its entire circumference. It would appear that the final act of engulfment was achieved with the aid of filopodia (Figure 3), and since the membrane component derived from these was not coated, a partially coated vacuole resulted from this process (Figure 4).

Thus partially coated vacuoles seem to represent a peculiar form of endocytotic process where both the invaginating mechanism of micropinocytosis and the enwrapping action of a fold or folds from the cell membrane play a part in impounding proteinaceous material from the extracellular environment. The significance of this phenomenon is obscure, but one may speculate that this could indicate either a heightened or speeded up endocytotic activity, or that this represents a defective micropinocytotic mechanism whereby the dense plaque fails to invaginate completely and the endocytotic act is completed by the process of pinocytosis.

While the formation of these partially coated vacuoles was easy to follow, their ultimate fate was difficult to trace with confidence. It seems to us that at some stage they lose the dense coat on the cytoplasmic surface and hence become difficult to identify. It is interesting to note that a similar observation was made by Roth and Porter[7] regarding the coated vesicles in the oocyte of *Aedes aegypti*, where also transitions from coated vesicles to

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yolk bodies was difficult to trace, presumably due to loss of the coat on the cytoplasmic surface.

However, since in our material numerous dense bodies acceptable as phagolysosomes (Figure 5) where fibrin seemed to be in the process of breakdown were present, we think that the partially coated vacuole is a type of phagosome by which the synovial cells of this patient were impounding fibrin from the joint space.

Zusammenfassung. Bei einem Individuum mit Multipler Sklerose, Psoriasis und Arthritis konnte man bei elektronenmikroskopischer Kontrolle von Synovialgewebe des Knies feine Filopodien nachweisen, die wahrscheinlich an der Aufnahme von Zellfragmenten, Fibrin und Erythrozyten im Gelenkspalt beteiligt sind.

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[8] Work supported by the Medical Research Council of Canada and the Canadian Arthritis and Rheumatism Society.

Biogenic Amines in a Retransplantable Neurogenic Teratocarcinoma

Monophyletic neurogenic teratocarcinomas are tumors composed of embryonal carcinoma cells, which are the stem cells of the tumor, and neuroectodermal tissues derived from the stem cells[1]. Despite the fact that this neuroectodermal tissue stems from rapidly proliferating malignant cells, it resembles in many respects the normal neural tissue derived from normal embryonic cells[1,2]. In this study we have analysed the neurogenic teratocarcinomas for their content of biogenic amines, known to have a specific role in neurotransmission, in order to get some insight into the functional status of this peculiar tumor.

Materials and methods. A retransplantable neurogenic teratocarcinoma, described in detail elsewhere[1], was obtained upon retransplantation of murine embryo-derived experimental teratocarcinomas. Tumor was histologically composed of embryonal carcinoma cells and neural tissue of varying degrees of maturity as determined histochemically and ultrastructurally[1]. Maturity of various elements of the tumor was, however, predominantly on the cytological level and the overall histological organization of the tumor was rather low. Neural tissue in the tumor did not mimic any organized neutral structure except embryonic neural tubes. Synapses and gliovascular junctions were noticed as the signs of the highest structural organization.

Tumor-bearing mice were decapitated, their tumors and brains removed, washed in ice cold saline, dried with filter paper and stored at 15 °C until the next day, when biochemical analyses were performed. 5-Hydroxytryptamine (5-HT) and 5-hydroxyindolacetic acid (5-HIAA) were extracted and spectrofluorometrically determined according to the method of CURZON and GREEN[3]. Noradrenalin (NA) and dopamine (DA) were also determined spectrofluorometrically using a slight modification of the method described by LAVERTY and TAYLOR[4]. Identification of each substance investigated was performed by recording their excitation and fluorescence spectra. In addition to untreated animals, an experimental group of 4 tumor-bearing animals received 25 mg/kg body weight of trancylpromine (Parnate, SK & F–385) i.p. 1.5 h prior to decapitation. Another group of 4 animals bearing neurogenic teratocarcinomas was given probenecid in a dose of 200 mg per kg body weight by single i.p. injection 1.5 h prior to decapitation.

Results. As shown in the Table, the tumors examined contained 5-HT and 5-HIAA in amounts comparable or even higher than those measured in normal mouse brains. There was, however, much more variation in the content of these substances in tumors than in the brains. It is notable that the tumors did not contain NA and DA in measurable amounts.

Treatment of tumor bearing animals with trancylpromine caused an increase of 5-HT in tumors for 29% ($P < 0.05$) and a decrease of 5-HIAA for 60% ($P < 0.01$) in relation to control values. Injection of probenecid to tumor-bearing animals caused no detectable increase in the content of 5-HIAA in the tumors.

Discussion. Our data show that neurogenic teratocarcinomas contain 5-HT and 5-HIAA, despite their structural immaturity on the histological level. Remarkable is, however, the absence of NA and DA in the tumors.

It is known that embryonal neural tissues contain less 5-HT and 5-HIAA than the adult ones[5]. The data obtained on teratocarcinomas indicate that histologically immature neural tissues do not necessarily have lower amounts of 5-HT and 5-HIAA than normal adult brain if the cells forming those tissues have attained full cytologic maturity. It is also evident that the neural tissues derived from malignant stem cells do not lack

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Content of 5-HT, 5-HIAA, NA and DA in the brain and the neurogenic teratocarcinoma of mice

	5-HT	5-HIAA	NA	DA
Brain	637 ± 37 (6)	302 ± 26 (6)	264 ± 13 (4)	458 ± 42 (4)
Tumor	1123 ± 304 (6)	276 ± 71 (6)	0 (4)	0 (4)

Values are given in nanograms per gram of tissue and represent the means ± S.E. Number of animals is given in brackets.