osmiophilen Inhalt. Dies deutet auf eine Aktivität des Golgi-Apparates hin. Berücksichtigt man die Übergangsstadien zwischen den einzelnen Granulaformen, so könnte man darin eine Entwicklungsreihe sehen. Die homogenen, osmiophilen Körper könnte man mit dem α-Granulomer der Blutplättchen des Menschen[13] vergleichen. Dies steht im Gegensatz zur Beobachtung SCHUHMACHERS[7], der ihr Vorkommen überhaupt ausschloss. Die lamellären und vakuolären Körper dürften den lichtmikroskopisch nachgewiesenen Zytosomen entsprechen[10]. Lamellenkörper wurden auch schon in anderen Zellen z.B. in Blutplättchen von Menschen[12] und Hunden[14] oder Leberzellen von Ratten[15] gefunden. Sie werden als ein spezielles Zwischenstadium im Kohlenhydrat- und Fettstoffwechsel[15] oder als Degenerationserscheinungen[14] angesehen. Myelinmembranen wurden ebenfalls an der Peripherie osmiophiler Granula und Vakuolen der Blutplättchen des Menschen festgestellt[12]. Sie werden als Sitz von Gerinnungsfaktoren diskutiert[12].

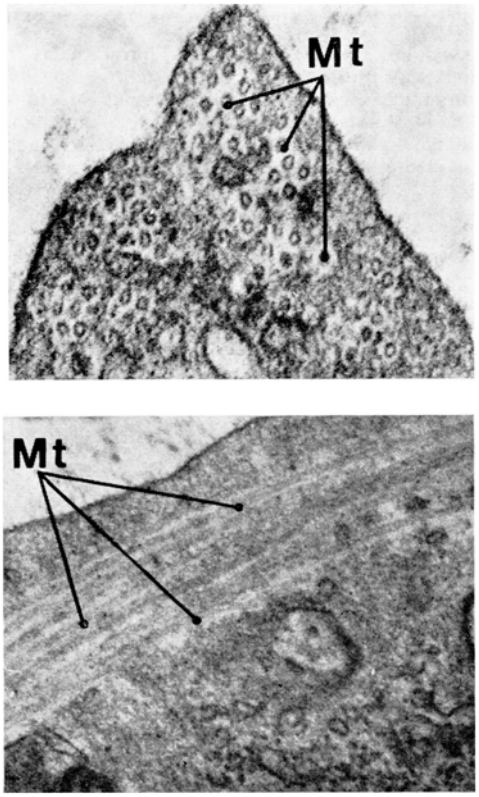

Fig. 2a. Mikrotubuli (Mt), quer; b. Mikrotubuli (Mt), längs. × 90000.

Entgegen der Feststellung SCHUHMACHERS[7] befinden sich im Zytoplasma weiterhin feine Röhrchen (Mikrotubuli) (Figur 2a, b), die mit denen der Thrombozyten des Menschen[1,3], der Amphibien[4] und der Fische[4,16] vergleichbar sind. Sie verlaufen in der Nähe der Zellmembran (untereinander parallel) und sind teilweise zu Bündeln angeordnet. Ihr Durchmesser beträgt 150–200 Å, ihre Wandstärke 50–80 Å. Sie waren nicht in allen Zellanschnitten zu sehen; das mag auf ihren Verlauf zurückzuführen sein, könnte aber auch Folge einer gewissen Labilität der Tubuli sein. Ihre Lage und die Tatsache, dass in einem bestimmten Plasmaareal entweder nur Längs-, Quer- oder Schrägschnitte auftreten, lassen darauf schliessen, dass sie die Zelle ringförmig durchziehen. Möglicherweise bilden sie auch hier – ähnlich wie bei den Blutplättchen des Menschen[1,3], – ein geschlossenes System.

Summary. Thrombocytes of clinical healthy, laying hybrid-hens were studied by using the electron microscope EM 9. In the cytoplasm of these cells osmiophilic granules of different stages were found, also lamellary bodies with or without vacuoles. A single lamella consists of myelin-membranes. Furthermore linear, branched out or circular membranes, which consist of 3 stratums, were observed in the cytoplasm. Ring-shaped microtubules lay close by the plasma membrane.

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[13] H. SCHULZ und E. HIEPLER, Klin. Wschr. *37*, 273 (1959).
[14] Z. BEN-ISHEY, J. WIENER, J. SWEETING, E. BADLEY und D. SPIRO, Lab. Invest. *17*, 190 (1967).
[15] A. CECIO, Z. Zellforsch. mikrosk. Anat. *62*, 717 (1964).
[16] D. SHEPRO, F. R. BELAMARICH und R. BRANSON, Anat. Rec. *156*, 203 (1966).

RNA-Induced Immunity Against a Rat Sarcoma

The transfer of acquired immunity against experimental tumours or leukemias has been studied by several authors. Recently, it has been reported that the injection of spleen cells or lymphocytes from immune donors into normal animals either delayed the growth of transplantable tumours or leukemias or induced their complete rejection[1–4]. COHEN[5] and FRIEDMAN[6] obtained in normal spleen cells an immune reaction against foreign antigen in vitro by addition of RNA from immune spleen cells. ALEXANDER et al.[7] reported the inhibitory effect of nucleic acids from rat and sheep immune lymphocytes on rat sarcomas.

The purpose of this work was the study of the immunity against a rat sarcoma (Sarcoma E 100) induced by RNA from immune rats. This study was started independently from that of ALEXANDER et al.[7] and used a different experimental model: the tumour is a transplantable sarcoma maintained in inbred rats and the RNA from the immune donor is injected only once, 5 days prior to the grafting of the tumour.

Sarcoma E 100 appeared spontaneously[8] in our rat colony in 1955 and is maintained by s.c. passages in an inbred line (F = 0.95). The percentage of 'take' of the first graft is 95%. To induce immunity the tumours of the first transplantation are excised and the animals regrafted 3 times. The second graft has a lower percentage of 'take' (33%) and the third and fourth grafts take at rates of 6 and 4% respectively. All the tumours that grow are

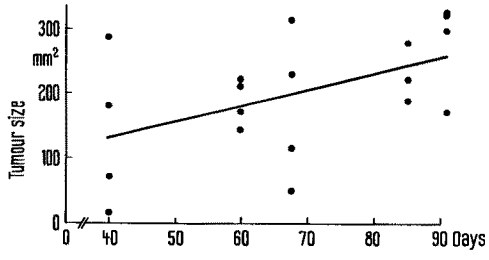
surgically excised and it has been observed that after the fourth passage the animals reject them invariably. RNA was extracted from the spleen of these immune rats and from normal rats. The extraction was performed by the phenolic fractionation method described by GEORGIEV et al.[9], the RNA used was that obtained at room temperature in the first part of the procedure. The immune and normal RNA were diluted in 0.14 *M* sodium chloride and each animal received the RNA contained in one spleen (approximately 4 mg/100 g of body weight). The rats were injected i.p., and 5 days later the 2 groups with normal and immune RNA, and a third one without RNA were challenged with Sarcoma E 100. The tumour growth was measured with calipers and the result was expressed in mm^2 (large diameter × minor diameter), for the correlation between this measurement and the tumour weight is very high ($r = 0.95$, $p < 0.001$). Adult rats of both sexes were used. They belonged to 3 different inbred lines and to an F_1 cross (a, g, e′ and ad)[10]. The tumour was always maintained in the same line (a), but RNA was extracted from any one of these lines. In spite of minor differences of histocompatibility, Sarcoma E 100 grows well in all these lines.

At first, the immune RNA extraction was performed at different periods of time after the last immunization passage. The inhibitory effect on the growth of the sarcoma decreased as the time increased. The correlation between both variables was significant ($r = 0.51$, $p < 0.05$) (Figure). Thereafter the immune RNA was always extracted 40 days after the last immunization (fourth).

The tumours were measured at 20 and 30 days after grafting. Immune RNA inhibited the tumour growth at 20 days, and this inhibition increased at 30 days. The controls and the group that received normal RNA did not differ, so they were pooled (Table). Though the tumours grew less in immune RNA-treated rats, they were seldom finally rejected and eventually killed the animals.

The RNA injected contained 10% protein and 10% DNA. Proteins cannot be the main cause of the inhibition, because no circulating antibodies are operative against this tumour according to previous unpublished results. Concerning DNA, it seems, on logical grounds, that its influence is unlikely, although it cannot be dismissed.

As an explanation of this phenomenon, a working hypothesis is proposed. The exogenous RNA carrying the 'immune message' is injected in a small and single dose 5 days before the tumour challenge; it is then the first information which the animal receives concerning the tumour. This RNA in some way acts like a template that elicits or facilitates the release of the rat's own messenger RNA when the tumour antigen begins to work. In this way, the immune RNA is instrumental in evoking a rapid response, thus probably transforming a first set immunological reaction into a second set one. This interpretation would also explain why the coded message contained in the immune RNA passes the strain and even the species barrier[7], since the experimental animal would only derepress the information related to those stimuli which it recognizes as antigenic, that is non-self – in this particular case, the tumour antigen[11].



Abscissa: Days from the last passage of the tumour in the donors of immune RNA. Ordinate: Size of the tumour at 20 days after transplantation in immune RNA treated rats.

Effect of immune and normal RNA on the growth of the tumour

Treatment	Time elapsed after grafting: 20 days No.	$\bar{x}$	P	30 days No.	$\bar{x}$	P
Immune RNA	22	151.3		17	365.1	
Normal RNA	19	227.0	<0.05	15	627.4	<0.05
Controls	20	238.1	<0.05	12	664.2	<0.01
Normal RNA and controls	39	232.7	<0.01	27	643.8	<0.01

Each group is compared with the immune RNA. Tumour size is expressed in mm^2. P values are obtained from an analysis of variance.

Resumen. Se estudió el efecto del ARN proveniente del bazo de ratas inmunizadas a un sarcoma transplantable (Sarcoma E 100) en el crecimiento del mismo. Se inyectó ARN normal y ARN de ratas inmunizadas a dos grupos de animales 5 días antes de la implantación del tumor, tomándose un tercer grupo como testigo normal. Los animales que recibieron el ARN inmune tuvieron tumores más pequeños que los de los dos testigos ($p < 0.01$), a los 30 días después del injerto. Se formula la hipótesis de que el ARN inmune posee la información (memoria inmunológica) para transformar una respuesta inmunitaria primaria en secundaria.

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[1] E. J. DELORME and P. ALEXANDER, Lancet *2*, 117 (1964).
[2] Z. B. MIKULSKA, C. SMITH and P. ALEXANDER, J. natn. Cancer Inst. *36*, 29 (1966).
[3] P. ALEXANDER, E. J. DELORME and J. G. HALL, Lancet *1*, 1186 (1966).
[4] P. ALEXANDER, D. I. CONNELL and Z. B. MIKULSKA, Cancer Res. *26*, 1508 (1966).
[5] E. P. COHEN and J. J. PARKS, Science *144*, 1012 (1964).
[6] H. FRIEDMAN, Science *146*, 934 (1964).
[7] P. ALEXANDER, E. J. DELORME, L. D. G. HAMILTON and J. G. HALL, Nature *213*, 569 (1967).
[8] A. D. DOMÉNICO, S. L. RABASA, M. T. FONT and J. M. SUÁREZ, Cienc. Invest. *19*, 462 (1963).
[9] G. P. GEORGIEV, O. P. SAMARINA, M. I. LERMAN, M. N. SMIRNOV and A. N. SEVERTZOV, Nature *200*, 1291 (1963).
[10] S. L. RABASA and M. T. FONT, Cienc. Invest. *19*, 133 (1963).
[11] This work was supported in part by a grant from the Consejo Nacional de Investigaciones Científicas y Técnicas.

Positive Immunological Reaction of Gut Cells from Orally Immunized Animals Demonstrated by Passive Cutaneous Anaphylaxis

The oral immunization of animals as described in various papers by RAETTIG[1,2] and resumed in a series of 5 papers[3–7], always lacked the demonstration of adequate antibody level or immunologically competent cells. In spite of a great protection index in mice[3,5,7] and during an epidemic[1], achieved by orally introduced antigens, it was only possible by the hemagglutination technique to demonstrate the presence of low antibody titers.

ROSENBERG, CHANDLER et al.[8] demonstrated the possibility of showing immunologically active cells when these were implanted intracutaneously into normal recipients, and the possibility of detecting the presence of antibody by inducing the passive cutaneous anaphylaxis (PCA) reaction with excess of antigen and dye as indicator. This communication presents results obtained with gut cells, from orally immunized guinea-pigs, implanted in recipients, guinea-pigs and rats, and challenged with the antigen used for immunization.

Twenty guinea-pigs of Pirbright white W.58 strain were orally immunized with a heat inactivated (100 °C for 5 min) germ suspension[7] containing a mixture in equal parts of *Salmonella typhi murium* strains 6616, 6639, 6643, 6656, 6659 RKTCC, total germ cell count was 10^9 ml. The animals were fed with 1 ml of this suspension every day for 10 days.

Sixteen days after the last immunization dose the gut and spleen were removed and prepared for the PCA technique. Gut and spleen pieces were gently homogenized with a micro tissue grinder in a chilled Eagle medium. The suspension was filtered through a cheesecloth and the nucleated cells counted in a hemocytometer chamber by adding 10% of a Giemsas 1% solution in 1 *M* citric acid. Adequate quantity of cell suspension were obtained by dilution or gentle centrifugation at 4 °C for 5 min at 1500 rpm and resuspension in an adequate volume.

The animals used as recipients were normal Pirbright white W.58 strain guinea-pigs weighing 250 ± 50 g and normal white rats weighing 200 ± 50 g. Each animal was sensibilized i.c. into the flank at 3 or 4 points. Volumes of 0.1–0.3 ml were injected with a needle 20 (0.45 × 21 mm) short bevel. A maximum of 30 min elapsed between excision of the donor tissues and injection of the cell suspension into the recipients. The animals were challenged in triplicates at the following times: 2, 24, 48, 96, 120 h. The antigen for challenge was prepared by freezing and thawing the germ cell suspension (10^9 cells ml) until no more living cells were present as proved by the sterility test. The challenge i.v. injection contained 0.5 ml of this antigen and 0.5 ml of a 0.5% Evans blue solution in saline. The guinea-pigs were challenged through the ear vein and the rats through the tail vein with a 0.30 × 16 mm needle. 20 min after challenge recipients were sacrificed. The skin was inverted and sites were examined for the dye location, the lesions diameter was measured with a transparent ruler. Gut and spleen cells from non-immunized guinea-pigs were used as control.

As can be seen from the Table, the implanted gut cells are able to evidence a positive PCA reaction after more than 24 h, with a maximum reaction between 72 and 96 h. The reaction when challenged 2 h after sensibilization is negative in all instances. The intensity of the reaction is

1 H. RAETTIG, Z. ImmunForsch. exp. Ther. *108*, 165 (1950).
2 H. RAETTIG, Zentbl. Bakt. ParasitKde *183*, 1 (1962).
3 H. RAETTIG, Zentbl. Bakt. ParasitKde *193*, 398 (1964).
4 H. RAETTIG, Zentbl. Bakt. ParasitKde *197*, 368 (1965).
5 H. RAETTIG, Zentbl. Bakt. ParasitKde *203*, 478 (1967).
6 H. RAETTIG and A. BUSE, Zentbl. Bakt. ParasitKde *204*, 221 (1967).
7 B. LIETZ and H. RAETTIG, Zentbl. Bakt. ParasitKde *204*, 543 (1967).
8 L. T. ROSENBERG, M. H. CHANDLER and E. E. FISCHEL, J. Immun. *81*, 136 (1958).

Immunological reaction of cells from orally immunized animals

Donor of cells			Recipient				Interval between transfer and challenge, h					
Species of animal cell donor	Antigen orally introduced	Interval between last oral immunization and gut removal Days	Species of animal cell receptor	No. of animals	No. of cells injected	Total sides sensibilized	2	24	48	72	96	120
GP	STM	16	GP	4	1.5×10^6	12	O	++	+++	++++	++	O
GP	STM	16	GP	4	1×10^6	12	O	+	+++	++++	±	O
GP	STM	16	GP	4	1.5×10^5	12	O	+	++	+++	+	O
GP	STM	16	GP	4	1×10^5	12	O	±	++	+++	+	O
GP	STM	16	GP	4	1.5×10^4	12	O	O	+	+	O	O
GP	STM	16	GP	4	1×10^4	12	O	O	±	O	O	O
GP	STM	16	R	4	1×10^8	12	O	±	+	++	+	O
GP	STM	16	R	4	1×10^7	12	O	±	+	+	O	O
GP	STM	16	R	4	1.5×10^6	12	O	O	O	O	O	O
GP	STM	16	R	4	1×10^6	12	O	O	O	O	O	O
GP	STM	16	R	4	5×10^5	12	O	O	O	O	O	O
GP	STM	16	R	4	1×10^5	12	O	O	O	O	O	O

GP, guinea-pig; R, rat; STM, heat inactivated *Salmonella typhi murium*; O, no reaction; +, less than 5 mm ϕ; ++, 5–10 mm ϕ, +++, 10–15 mm ϕ.