trated. Particular attention has been drawn to variations in these features and to those aspects that differ from those found in humans. In particular, retrograde mesenteric lymphatic backflow is common when these animals are examined lying on their right side.

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References

1 *Greene, E. C.:* The anatomy of the rat. Hafner Publishing Co., New York 1963
2 *Farris, E. J., J. Q. Griffith:* The rat in laboratory investigation. Hafner Publishing Co., New York 1963
3 *Kreel, L., S. Raia:* The rat as experimental model in sequential radiologic study of the thoracic duct. Invest. Radiol. 4 (1969), 50-53

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Influence of Neonatal Thymectomy on the Development of Primary Rous Sarcomas in two Inbred Strains of Rats*

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In a number of experimental systems it has been shown, that neonatal thymectomy renders the animals more susceptible to the action of oncogenic viruses – polyoma virus in mice (7) and rats (10), SV 40 virus in rats (2), Rous sarcoma virus in chickens (8).

Tumour specific transplantation antigens have been demonstrated in mice (5) and in rats (4) carrying sarcomas induced by Rous sarcoma virus of the *Schmidt-Ruppin* strain (RSV-SR). It was the aim of the present investigation to study the role of the immunologic impairment following neonatal thymectomy for the course of development of primary sarcomas induced by RSV-SR in two inbred strains of rats.

In the search for suitable experimental animals five strains of rats were studied in advance for the effect of neonatal thymectomy upon their lymphoid tissue and their immunological capacity (3); neonatally thymectomized and sham-operated rats were immunized with sheep red blood cells when adult, and their haemolysin titers measured; also the cell content of particular lymph nodes was determined. A significant reduction in haemolysin titers and of cell counts of lymph nodes in the groups of neonatally thymectomized animals as compared with their sham-operated litter mates was observed in the inbred Wistar/Fu (W/Fu) and Black/Norway (B/N) strains of rats. Therefore these two strains were selected for the present long term experiment.

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Material and Methods

Rats from 11 litters of the inbred W/Fu strain and from 12 litters of the inbred B/N strain were thymectomized or sham-operated within the first 24 hours after birth. As far as possible each litter was divided in an equal number of thymectomized and sham-operated males and females.

Operating Procedure

Anaesthesia was cooling for about 20 minutes in the refrigerator. An incision was made in the skin in the midline, and with a corneal scissor the sternum was cut to the level of the second costa. The layer of pretracheal muscles was split in the midline and the upper part of the thymus gland was thus exposed. The cranial parts of the thymus lobes were gently freed from the underlying tissue. In the case of sham-operation the incision was closed at this stage. In the case of thymectomy the thymus was sucked out through a glass tube and the organ blowed out in a Petri dish with water. Inspection of the thymus gland in the dish determined whether the operation was successful or not: did the thymus look incomplete and teased, remaining thymic tissue was searched for in the thorax. Did it look complete, the incision was closed at once with three silk sutures in the skin. Nobecutan® was sprayed upon the cicatrice. When the animals were again warm they were given back to their mothers.

At the age of four weeks the animals were weaned and separated in cages according to sex. At the same time they received one injection of 0.2 ml in the right back leg of RSV-SR from the same pool, which had a titer of 5×10^4 FFU/ml. The virus pool was kept at minus 70 centigrades and was thawn immediately before use. The injection was given subcutaneously, but the needle was inserted through the muscle. Once a week the animals were inspected and palpated for possible formation of tumours and cysts.

The animals were killed when the tumour had ulcerated or grown so big as to cause mechanical discomfort to the rat. Tumours and cysts were examined by routine histology. At autopsy a final cheque was made as to whether the thymectomy was complete or not. If thymic tissue remnants were found in rats of the group of neonatally thymectomized, these individuals were excluded from the material which ended up with a total of 88 W/Fu rats and 59 B/N rats.

Results

Mortality due to operational failure was lower than five per cent. The mortality due to maternal neglect or cannibalism was somewhat greater, especially in the B/N strain. A syndrome like wasting disease was seen in only one animal.

Tumours arose at the site of injection of the RSV-SR in all animals. Most often they were first detected in the muscle along the insertion canal of the injection needle as one or more small nodules. When the tumours grew big, the interior part often necrotized; this sometimes resulted in open ulceration. The histological picture was in all cases that of fibrosarcomas, more or less differentiated.

In addition to solid tumours cysts sometimes developed at the site of RSV-SR injection or in other locations, the most frequent being retroperitoneally in the pelvis. The cyst

formation started in lymph nodes which could be transformed into large cavities filled with a serum-like or a haemorrhagic fluid.

Frequencies and Latency Periods for Tumour Formation

The results are summarized in table 1. All animals developed tumours at the site of RSV-SR injection whether they were neonatally thymectomized or sham-operated.

The latency period was not influenced by neonatal thymectomy in the group of male rats of the W/Fu strain, but in the group of female W/Fu rats it caused a decrease of four weeks in the average latency period. Rats of the B/N strain showed a reduction of seven to eight weeks in the average latency period as a result of neonatal thymectomy.

The frequencies of internal cysts and tumour metastases were higher among thymectomized male rats of the W/Fu strain as compared to the sham-operated individuals. The females were not affected by thymectomy in this respect. Both males and females of the B/N strain showed a somewhat higher frequency of cysts following neonatal thymectomy while the frequency of metastases was unaltered.

Table 1 The Influence of Neonatal Thymectomy on the Development of Rous Sarcomas in two Inbred Strains of Rats.

	Males		Females	
	thymect.	shamop.	thymect.	shamop.
Rats, W/Fu strain:				
Frequency of tumour formation	25/25	22/22	20/20	21/21
Latency period (weeks)	13.0	12.8	11.3	15.5
Frequency of cysts in situ	2/25	5/22	6/20	4/21
Frequency of cysts in other locations	11/25	4/22	8/20	10/21
Frequency of tumour metastases	13/25	5/22	11/20	11/21
Rats, B/N strain:				
Frequency of tumour formation	17/17	14/14	14/14	14/14
Latency period (weeks)	24.6	31.9	30.6	38.6
Frequency of cysts in situ	5/17	1/14	2/14	1/14
Frequency of cysts in other locations	4/17	1/14	3/14	0/14
Frequency of tumour metastases	0/17	1/14	2/14	1/14

Fig. 1 represents the cumulative frequencies of tumours as a function of time in the four groups of W/Fu rats: neonatally thymectomized and sham-operated males and females. It appears that the course of tumour formation was not much different between the groups; the only minor difference was that some late tumours arising in the group of sham-operated female rats appeared to be accelerated in the group of thymectomized female rats.

Fig. 2 illustrates cumulative tumour frequencies among B/N rats. Neonatal thymectomy provoked in this strain an early wave of tumour formation about the 10th week after RSV-SR inoculation. A second wave of tumour development from the 30th to the 35th week after RSV-SR infection appeared to be more or less alike in thymectomized and sham-operated animals.

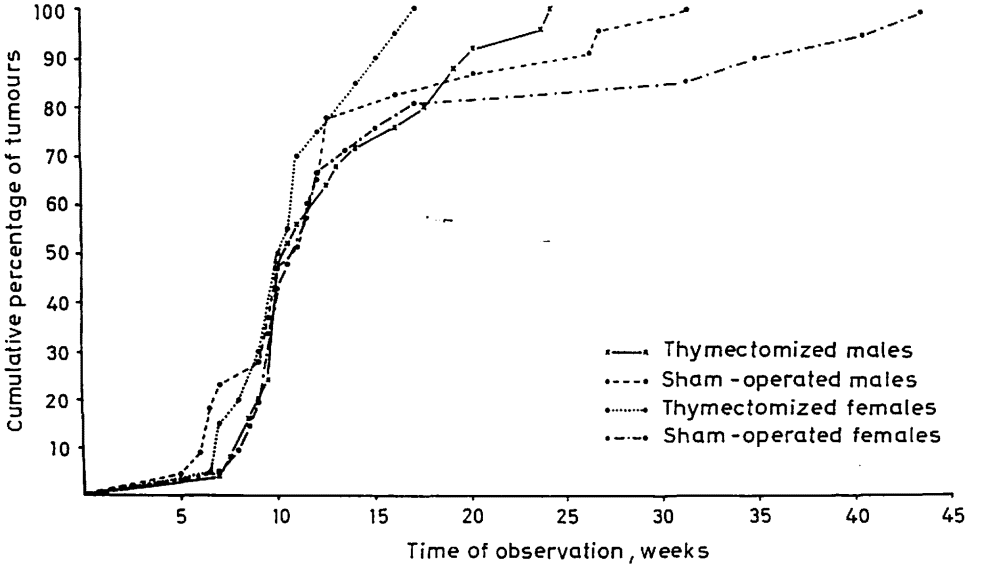

Fig. 1 Cumulative percentage of tumours arising in neonatally thymectomized and sham-operated W/Fu rats following one injection of RSV-SR at the age of four weeks.

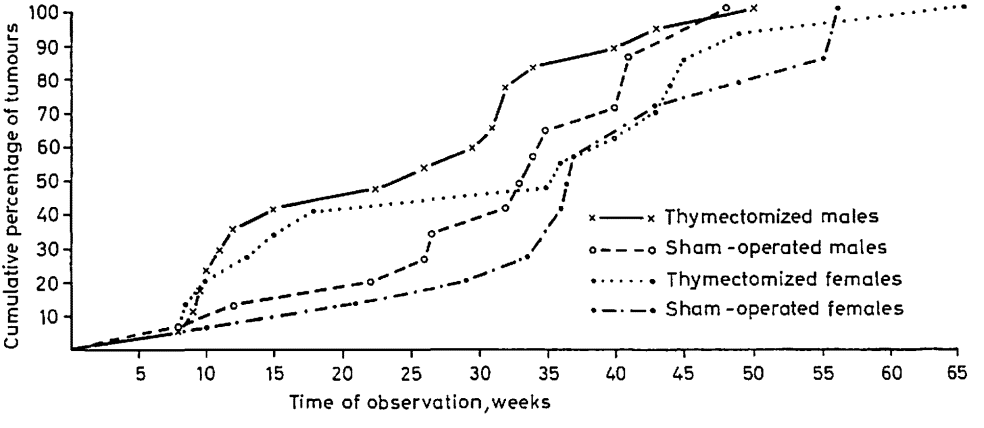

Fig. 2 Cumulative percentage of tumours arising in neonatally thymectomized and sham-operated B/N rats following one injection of RSV-SR at the age of four weeks.

Discussion

In the W/Fu strain of rats neonatal thymectomy caused a three to four times reduction of the primary haemolysin response following challenge with sheep red blood cells and a three to four times reduction of the cell content of the regional lymph nodes; in the B/N strain there was 1.5 to 2 times reduction of these values (3). The effect of neonatal thymectomy upon RSV-SR tumourigenesis in these rats was however modest,

and did in no way parallel the effects upon lymph node cell counts and haemolysin titers. On the contrary: the influence of neonatal thymectomy upon RSV-SR tumourigenesis was more marked in the B/N strain than in the W/Fu strain.

In contrast to the small effects of neonatal thymectomy in the RSV-SR system, neonatal thymectomy had a much greater influence upon polyoma tumourigenesis in these two strains of rats, where both tumour frequency and latency periods were substantially influenced (9).

There was a somewhat higher frequency of haemorrhagic cysts among neonatally thymectomized animals than among sham-operated litter mates. *Ahlström* and *Jonsson* (1) found in their study of Rous sarcoma induction in rats that cysts developed when RSV-SR was inoculated to newborn individuals but not when inoculation took place after the second week of age. It thus appears that the formation of haemorrhagic cysts in response to RSV-SR challenge is apt to take place in an immunologic immature or impaired animal.

The difference between the W/Fu and B/N strains in their response to the same treatment of RSV-SR is remarkable. While the W/Fu strain developed tumours from 13–16 weeks on the average, the B/N strain developed tumours from 25–39 weeks after virus inoculation. The formation of cysts and metastases was more frequent in the W/Fu strain. This strain thus appeared to be more susceptible to the action of RSV-SR as did the B/N strain. A similar difference between the same two strains in their response to polyoma virus infection was noted by *Vandeputte* (11) and by *Sjögren* and *Borum* (9).

It would be interesting to try to correlate the total amount of lymphoid tissue present in the two strains of rats with their resistance to RSV-SR challenge. Therefore eight individuals of each strain, four males and four females, were sacrificed at the age of 2 months and the thymus gland, the spleen and all lymph nodes except the mesenteric ones, were weighed and made into cell suspensions and their cell counts determined. The average values are given in table 2.

Table 2 Weight and cell content of the lymphoid tissue in 2 months old W/Fu and B/N rats.

Number of animals	Weight (grams)				Cell count × 10^7		
	body	thymus	spleen	lymph nodes	thymus	spleen	lymph nodes
4 W/Fu, ♂	160	0.32	0.42	0.19	68	33	3.5
4 W/Fu, ♀	120	0.26	0.32	0.20	45	19	6.1
4 B/N, ♂	165	0.30	0.43	0.37	57	47	25.0
4 B/N, ♀	120	0.26	0.30	0.21	42	29	32.0

As can be seen the main difference between the two strains in relation to the amount of lymphoid tissue was the cell content of the lymph nodes. In the B/N strain the lymph nodes contained 7 times (males) and 5 times (females) as many cells in peripheral lymph nodes than did the W/Fu strain. It is proposed as a possibility that the greater resistance to RSV-SR found in the B/N strain might be due to the presence of many more lymphoid cells in the peripheral lymph nodes in this strain as compared to the W/Fu strain.

Summary

Neonatal thymectomy or sham-operation was performed upon 88 rats of the inbred W/Fu strain and upon 59 rats of the inbred B/N strain. At the age of four weeks the animals received one injection subcutaneously in the leg of RSV-SR from the same pool, the titer of which was 5×10^4 FFU/ml.

The rats developed tumours in 100 per cent of the animals, whether thymectomy or sham-operation had been performed. In the W/Fu strain the group of neonatally thymectomized females showed a diminution of four weeks in the average latency period for tumour formation, while the latency period was alike among thymectomized and sham-operated males. In the B/N strain the influence of neonatal thymectomy manifested itself in a seven to eight weeks decrease in latency period, both among males and females. The incidence of cysts was somewhat higher among thymectomized individuals.

The average latency period for tumour development was 13–16 weeks in the W/Fu strain and 25–39 weeks in the B/N strain which then appear to be more resistant to the RSV-SR treatment. It is suggested that the greater resistance is related to the greater amount of lymph node tissue present in the B/N strain as compared to the W/Fu strain.

References

1 *Ahlström, C. G., N. Jonsson:* Induction of sarcoma in rats by a variant of Rous virus. Acta path. scand. 54 (1962), 145-172

2 *Allison, A. C., R. B. Taylor:* Observation on thymectomy and carcinogenesis. Cancer Res. 27 (1967), 703-707

3 *Borum, Kirstine:* The influence of neonatal thymectomy on the lymphoid tissue and the immune capacity in five strains of rats. Acta path. scand. 1972 (in print)

4 *Jonsson, N.:* Cell-mediated and humoral immune reactions against tumor-specific and viral antigens in relation to Rous sarcoma virus tumorigenesis in rats. Acta path. scand. 77 (1969), 753-755

5 *Jonsson, N., H. O. Sjögren:* Further studies on specific transplantation antigens in Rous sarcoma of mice. J. exp. Med. 122 (1965), 403

6 *Kirstein, R. L., A. S. Rabson, E. A. Peters:* Oncogenic Activity of Adenovirus 12 in thymectomized BALB/c and C3H/NeH mice. Proc. Soc. exp. Biol. Med. 117 (1964), 198-200

7 *Miller, J. F. A. P., R. C. Ting, L. W. Law:* Influence of thymectomy on tumor induction by polyoma virus in C57Bl mice. Proc. Soc. exp. Biol. Med. 116 (1964), 323-327

8 *Radzichovskaja, R.:* Effect of thymectomy on Rous virus tumor growth induced in chickens. Proc. Soc. exp. Biol. Med. 125 (1967), 13-15

9 *Sjögren, H. O., K. Borum:* Tumor-specific immunity in the course of primary polyoma and Rous tumor development in intact and immuno-suppressed rats. Cancer Res. 31 (1971), 890-900

10 *Van de Putte, M., P. Denys, L. Leyton, P. de Somer:* The oncogenic activity of polyoma virus in thymectomized rats. Life Sci. 7 (1963), 475-478

11 *Van de Putte, M.:* Personal communication, 1970

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