Homograft Response and Lymphocyte Level in Thymectomized and/or Bursectomized Ducks

The significance of the thymus for immunological reactivity as demonstrated by the consequences of neonatal thymectomy in mice and rabbits[1-3] led us to investigate the possibility of using transplantation of homologous or heterologous thymus and possibly even of bursa of *Fabricius* in neonatally thymectomized and/or bursectomized ducks as a means of modifying the specificity of their immunological reactivity. Ducks were chosen because the stage of functional maturity of their immune systems at hatching (as revealed by the experiments of the induction of immunological tolerance) seemed to provide a suitable model for such an alteration.

The main results of the first phase of experiments are reported here, namely those on the effect of extirpation of thymus and/or of bursa of *Fabricius* on the homograft reaction and on the level of lymphocytes in the peripheral blood; more details of this work together with the results of its second part (transplantation of homologous thymus or bursa which caused skin test grafts to be tolerated for more than 40 weeks at the time of writing) are going to be published elsewhere (Fol. biol., Praha).

The hormonal 'bursectomy' as used in chickens did not prove a strictly selective treatment, inhibiting to a varying degree the development of the lymphoid tissues generally[4,5] and various types of immune response[6-10]; we thus preferred the surgical alternative. Both bursectomy (as described by MUELLER et al.[6] for chickens) and thymectomy (consisting of blunt preparation and extirpation

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The effects of surgical thymectomy and/or bursectomy performed in newly hatched ducks

Group	Duck No.	Sex	Relative organ/body weight × 10⁴			Average % lymphocytes (small and large) at the given age (in days)								Skin graft survival time (days)
			Thymus	Bursa	Spleen	14		28		56		70		
						S	L	S	L	S	L	S	L	
Controls	260	♀	22.3	13.3	6.2									14
	262[a]	♀	18.1	9.8	5.1									16
	264[a]	♂	16.7	7.8	5.0									15
	266[a]	♂	21.7	11.0	5.6	43	10	50	9	47	9	48	7	19
	268[a]	♀	24.7	11.8	9.1	±	±	±	±	±	±	±	±	17
	270[a]	♀	41.8	12.5	6.6	2.9	3.4	3.7	1.3	1.7	1.5	2.9	1.0	12
	274	♀	23.3	7.8	4.7									11
	278[a]	♂	21.1	9.6	8.5									11
	282	♂	33.4	18.2	11.6									19
	Mean value ± standard error of the mean		24.8 ± 2.7	11.3 ± 1.07	7.0 ± 0.8									
Bursectomy	250	♂	22.9	2.6	6.7									12
	254	♂	23.0	4.2	5.2	45	8	40	8	47	7	49	7	14
	258	♂	27.1	2.2	5.6	±	±	±	±	±	±	±	±	12
	5023	♀	73.6	2.1	7.8	5.2	0.1	5.2	1.5	8.2	1.5	2.9	1.5	18
	5025	♀	37.7	1.8	6.6									
	Mean value ± standard error of the mean		36.8 ± 9.1	2.6 ± 0.4	6.4 ± 0.4									
Thymectomy	173	♂	1.3	9.0	6.7									12
	174	♀	0	7.8	6.3									17
	176	♀	19.6	10.2	5.7	45	8	41	9	44	7	49	5	11
	180	♂	0.8	10.0	7.6	±	±	±	±	±	±	±	±	14
	182	♀	0.2	9.6	6.1	3.1	0.9	3.3	0.9	3.6	1.2	3.9	0.5	13
	187	♂	2.5	6.3	4.2									11
	5029	♀	3.2	12.2	8.3									
	Mean value ± standard error of the mean		3.9 ± 2.6	9.3 ± 2.25	6.4 ± 1.6									
Bursectomy and thymectomy	172	♀	6.2	2.1	5.3									11
	177	♀	0	1.6	10.3	29[b]	7	37[b]	8	31[c]	13	43	6	20
	240	♂	0.2	2.3	5.7	±	±	±	±	±	±	±	±	14
	242	♂	0.2	1.5	5.1	4.6	1.1	2.5	1.3	5.5	3.1	4.6	0.4	12
	Mean value ± standard error of the mean		1.6 ± 0.1	1.9 ± 0.2	6.6 ± 1.2									

[a] Lymphocyte counts made only in birds denoted.
[b] Significantly lower than control ($P < 0.05$).
[c] Significantly lower than control ($P < 0.01$).

of separate thymic lobes) were performed in Peking bred ducklings within 36 h of hatching. Test skin grafting was carried out at the age of 25–35 days. The peripheral blood picture was made at intervals given in the Table. Differential cell count was made from 150–200 white blood cells classified as polymorphs, monocytes, small and large lymphocytes. The Table gives the average percentage of small and large lymphocytes from the total of white cells scored. The Student's *t*-test was used for the statistical evaluation of the results. All the ducks were killed at the age of 80 days, checked for possible remnants of the removed organs, and their weight, as well as that of their spleen, was recorded and expressed relatively to the body weight. The Table shows that the extirpation of the bursa of *Fabricius* and thymus led to a transient lowering only of the level of the small lymphocytes in the peripheral blood; in all the three experimental groups the survival of skin homografts from donors belonging to the same population falls into the range characteristic for non-treated controls, showing no effects of the removal of these organs. No higher mortality or reduction of the spleen or body weight in comparison with control birds was observed.

These results seem to be in contrast to those of SZENBERG, WARNER and BURNET[9, 10] who found that complete atrophy of the thymic cortex (which was produced in some chickens by their being treated as 12-day-old embryos with testosterone) caused skin homografts to be retained by such birds for prolonged periods, in each case until their death occurring at the latest 24 days after grafting. With regard to the fact that our surgically thymectomized ducks appear fully competent to mount a normal homograft reaction, we feel that the reasons for this difference should be considered. The hormonal treatment of chicken embryos strongly impaired the viability of the hatched birds; even though the histology of their grafts revealed a slight cellular infiltration, the birds were dying with homografts in place. May not this failure differ only quantitatively from the normally rapid homograft rejection in chickens submitted to a similar hormonal treatment which, however, resulted in the atrophy of the bursa only leaving the thymic cortex intact? The more severely affected chickens may not represent quite a suitable system for using the homograft reaction as a measure of their immune capacity for fatal runt syndrome is a more generalized condition which might influence the rate of this reaction even indirectly.

The surgical bursectomy and thymectomy appears to be of some advantage, although a complete removal cannot always be granted (see Table); as, however, the organs cannot be removed in this way until after hatching, the factor of time, when the effect may set in, is to be taken into account. The fact that in different animal species, or even different lines of the same species[11–14], the effect of the neonatal thymectomy considerably differs, might be in agreement with the BURNET's view[15] that the functional development (or maybe capacity) of the thymus within the perinatal period may vary. However, our finding of a normal capacity to reject homografts, as demonstrated in ducks surgically thymectomized at hatching, is not in accord with the BURNET's expectation that such capacity should be lost in similarly treated chickens. As the development of the homograft-rejecting capacity in newly hatched normal chickens is known to be on the average even more advanced than in ducklings, an inhibiting effect of the surgical thymectomy can hardly be expected in the former. We intend, therefore, to follow the effect of removing thymus and/or bursa of *Fabricius* in nidicolous birds which are at a lower stage of their individual development at hatching; should the developmental stage of their immune systems be correspondingly low, pigeons might, for example, be a favourable experimental subject of choice.

Zusammenfassung. Der Einfluss der Exstirpation von Bursa fabr. und Thymus junger Enten wurde zur Erklärung der Bedeutung dieser Organe für die Entwicklung der immunologischen Reaktivität untersucht.

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Effect of Thiourea Treatment on Temperature Selection and Swimming Behaviour in *Phoxinus phoxinus*

Groups of *Phoxinus phoxinus* were immersed in thiourea (0.5 g/l) or aerated water (controls) and kept at either 20°C (±1°C) or 12°C (average aquarium temperature, range 9–15°C) for four weeks. Temperature selection tests followed.

These tests were carried out in an aluminium tank, heated at one end and cooled at the other, containing water to a depth of 5 inches. Three perspex baffles with large central holes were placed at equal distances along the tank. This system gave a horizontal gradient of about 5°C; the range of the gradient could be adjusted and two (19–23°C and 25–30°C) were used.

Single fish were placed centrally in the tank and a period of 2 h allowed for recovery. Positions were then recorded every 30 sec for 5 min in every quarter of an hour. Observations were continued for 2 h (thus giving a total of 80 positions) or until there had been no change in position for four sets of readings. Results were expressed as a percentage of the total number of observations. Ten tests were made for each group of fish; each of the ten being carried out with a different individual. The rate of change of position also gave an indication of the irritability of the fish and this was recorded as percentage number of times in which the fish remained stationary.

In the tank a fish would remain motionless for a few seconds and then begin to move rapidly and erratically. After a variable period (always less than the 2 h allowed) the fish settled down and became either stationary or exhibited only occasional movements. Fish treated with thiourea were more sensitive to disturbance and moved in short, erratic bursts.

All fish showed the same response when tested at the lower temperatures and selected the highest temperature available; results for those acclimatized at 12°C shown in