cells exhibiting nuclear localization were compared by positioning a reticule square comprising an area of 72 μm^2 over the cell nucleus and counting under the microscope at a magnification of $\times 580$ all the silver-grains contained in and touching 2 sides of the square. The dimensions of the square were chosen because they usually corresponded to the area occupied by the silver grains associated with the nuclei. In all instances adjacent areas outside the cells, occupied solely by neuropil were counted as well for comparison and the results are displayed since these counts always greatly exceeded the background count for the emulsion which was negligible (less than 1 silver grain per 1000 cm^2).

Results and discussion. As seen from a comparison of photomicrographs depicted in figure 1 and of counts of silver grains expressed per 100 μm^2 area in figure 2, the greatest concentration of silver grains occurred over the cell nuclei of the indusium griseum and hippocampus anterior, the arachnoid, and over motor components of the cranial nerves. Distinct, although less intense nuclear localization was noted in the post-commissural hippocampus and dentate gyrus, the anterior olfactory nucleus and in the reticular formation. No nuclear accumulation of silver grains was observed in any of the regions exposed to a 100-fold excess of unlabelled aldosterone before administration of the tritiated compound (range of counts 0–3 grains/1000 μm^2).

In the preference for motor over sensory components of the cranial nerves aldosterone resembles both the androgen steroid hormones and corticosterone but differs from estradiol[3]. It further differs from the sex hormones in its failure to localize in the area postrema and locus ceruleus. Our distribution studies extend the recent findings of Ermisch and Rühle[11] and provide quantitative evaluation of regional differences in the intensity of nuclear localization.

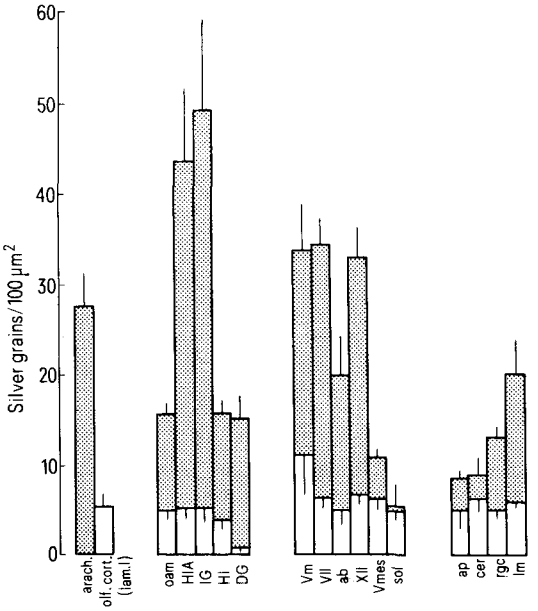

Fig. 2. Concentration of radioactivity in different brain regions, expressed per 100 μm^2 area and assessed by counting the silver grains in a 72 μm^2 grid positioned over the cell nucleus (stippled bars) or over adjacent neuropil (open bars). Vertical lines denote SEM (N = 5–10). arach = arachnoid over olfactory cortex (olf. cort.): oam = nucleus olfactorius anterior; HIA = hippocampus anterior; IG = indusium griseum; HI = hippocampus; DG = dentate gyrus; Vm, VII, ab, XII = motor nuclei of the 5th, 7th, 9th and 12th cranial nerves; Vmes, sol = sensory nuclei of the 5th and 10th cranial nerves; ap = area postrema, cer = locus ceruleus; rgc = nucleus reticularis gigantocellularis; lm = nucleus reticularis lateralis magnocellularis.

The functional significance of these target sites in the brain for aldosterone is not known nor does the autoradiographic evidence to date permit definite conclusions regarding receptor specificity. While similarities in the regional distribution of aldosterone and corticosterone are suggestive of common binding sites, the profiles for variations in regional intensities did not coincide and the possibility of separate specific receptors, located within the same cell or in adjacent cells, capable of discriminating between steroid configurations imparting glucocorticoid and mineralocorticoid properties to the molecule is not ruled out. Ongoing comparative autoradiographic studies on the localization of aldosterone and corticosterone following the administration of competing steroids of either category should shed light on this problem. While aldosterone is quantitatively only a minor secretory product in the rat, the structurally related mineralocorticoid 18-hydroxydeoxycorticosterone which also contains an oxygen function at carbon 18 can be secreted by this species in quantities exceeding those of corticosterone. In contrast to corticosterone the C-18 oxygenated adrenocortical steroids have only a minimal affinity for the corticosteroid binding globulin in plasma, a feature that might render them more accessible to target organs. Aldosterone is capable of inhibiting the stress-induced rise of plasma corticosterone in the rat, a property usually associated with glucocorticoids, at $^1/_{10}$ the concentration at which corticosterone itself is effective[12,13]. This might implicate primitive allocortical structures which appear to be even more conspicuous targets for aldosterone than for corticosterone as relevant sites for negative feedback action, a consideration rendered attractive by their projection to the hypothalamus. It is noteworthy that nuclear localization of aldosterone, and also of corticosterone occurred in the reticular formation which is considered an anatomical focus for arousal behaviour. Radiochemical evidence indicates that deoxycorticosterone, another potent natural mineralocorticoid capable of suppressing stress-induced elevation of corticosterone levels in the rat[14], locates in the reticular formation as well[15]. The concentration of aldosterone in cell nuclei of the arachnoid may be of significance in its therapeutic effect on cerebral edema[16]. The implications of the radioactivity associated with the neuropil also remain to be assessed.

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Studies on experimental ancylostomiasis: Transfer of acquired immunity to *Ancylostoma caninum* in mice through sensitized thymus and bone marrow cells

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Summary. An attempt has been made to transfer acquired immunity to *Ancylostoma caninum* infective larvae from infected Swiss albino female mice to nonimmune, isologous recipients of same sex, through immunized thymus and bone marrow cells. Immunized cells from donors infected with a single high dose of 1000 larvae were found to be more immunocompetent than cells from donors infected with a single, but low dose of 500 larvae.

It has now been established that passive immunity to helminthic infections can be induced in the nonimmune recipients by the transfer of lymphoid cells from infected donors[2]. Cells used for this purpose have usually been obtained from lymph nodes, peritoneal cavity, spleen and in a few cases from bone marrow.

The thymus has the greatest lymphopoetic rate in the body and is essential for a normal concentration of lymphocytes in blood, spleen and lymph nodes[3]. It is the site of differentiation of immunocompetent cells, receiving stem cells from the bone marrow which multiply extensively and differentiate to small lymphocytes, many of which are then seeded out into the circulation and join the pool of lymphocyte present in the peripheral lymphoid organs[4]. Most of the lymphocytes, however, die locally without subserving any obvious immunological function[5]. Thymus-dependent, recirculating and long-lived small lymphocytes have been demonstrated to be responsible for initiation of rejection of *Trichostrongylus columbriformis* in the guinea-pig[6]. Neonatal thymectomy had been found to cause delay in worm rejection (*Nippostrongylus brasiliensis* in rats[7]; *Hymenolepis nana* in mice[8,9]; *T. columbriformis* in guinea-pigs[6] and *Trichinella spiralis* in mice[10]). Restoration of immune response in thymectomised animals occurred when thymus tissues were retransplanted[9]. On the cellular basis, Cerottini et al.[11] have found that thymus cells on proper antigenic stimulation may differentiate into cells which display in vitro a cytotoxic effect on adequate target cells, i.e., into sensitized cells active in cell mediated immune response.

The bone marrow is also an immunologically competent tissue, though not as powerful as the spleen and lymph nodes. Lymphatic nodules have been reported in bone marrow which produce most of the antibody to locally administered antigens. It is the source of radiosensitive monocyte precursors which mature into monocyte macrophage cells required for the expression of the CMI[12,13]. A cell component derived from bone marrow has been found to play an important role in the expulsion of *N. brasiliensis* from rats[14] and *T. spiralis* from mice[15].

Cell-mediated immune response to *Ancylostoma caninum* larvae has been found to operate in pups[16] where transfered sensitized lymph node cells caused the expulsion of worms from the recipient pups. Vardhani and Johri[17,18] demonstrated that sensitized lymph node cells and peritoneal exudate cells from *A. caninum* infected donor mice are responsible for larval expulsion from syngeneic recipients.

The role of cells taken directly from the thymus and bone marrow of the infected donors during CMI has not been studied before. An attempt has been made in the present preliminary work to investigate whether cells from thymus and bone marrow of *A. caninum* infected female mice can cause expulsion of larvae from the recipients.

Material and methods. Isogenic female Swiss albino mice (6–8 weeks old, 20–23 g) were used in these experiments. Infective 3rd stage larvae of *A caninum* were obtained and administered following a modified method by Bhopale and Johri[19]. Donors were divided into 3 groups A, B and C each having 22 mice. Experimental donors of groups A and B were immunized by orally administering 1000 and 500 larvae respectively. Group C mice were not infected.

Cells were collected from the donors 21 days after infection using sterile technique. Intact thymuses were removed from donors after cervical dislocation and processed in Ringer's solution to release the cells. Bone marrow cells were collected from the humerus and femur of the same donors according to the method by Larsh et al.[20]. Approximately

Percentage of *A. caninum* larvae recovered initially at 6-h-intervals from recipient groups of thymus cells-a_1 (1000 larvae), b_1 (500 larvae), c_1 (control) and of bone marrow cells-a_2 (1000 larvae), b_2 (500 larvae) and c_2 (control) challenged with a single dose of 500 infective larvae 7 days after cell transfer. Readings are based on mean of recoveries made from 2 animals

Duration of infection at necropsy (h)	Group a_1 Mean value	χ^2-value (a_1 vs c_1)	Group b_1 Mean value	χ^2-value (b_1 vs c_1)	Group c_1 Mean value	Group c_2 Mean value	Group b_2 Mean value	χ^2-value (b_2 vs c_2)	Group a_2 Mean value	χ^2-value (a_2 vs c_2)
6	52.0	76.78	68.6	12.36	78.4	71.0	60.5	12.45	52.7	35.85
12	46.8	55.38	62.0	7.11	70.0	68.3	52.7	25.44	49.1	37.85
18	26.7	181.83	53.2	26.96	69.2	54.4	48.9	3.12	44.5	9.22
24	25.5	109.07	51.1	5.24	58.1	50.6	42.9	5.80	38.7	14.57
36	21.2	110.72	48.4	2.50	53.4	42.1	28.6	19.68	29.3	17.87
48	17.3	126.92	45.1	3.62	51.0	37.2	24.7	18.63	27.7	10.65
72	14.8	131.70	38.6	10.18	48.6	35.5	21.7	23.42	24.8	14.36
96	14.0	109.28	36.0	6.66	44.0	32.9	18.2	27.98	16.3	37.35
120	13.8	84.83	33.6	3.88	39.6	31.9	17.1	28.29	0.0	32.72
144	0.0	220.06	29.0	5.94	36.2	26.9	13.2	28.70	0.0	39.53
168	0.0	207.72	25.8	8.78	34.5	25.2	6.1	69.99	0.0	144.17
240	0.0	193.32	24.6	10.25	32.6	17.1	0.0	94.10	0.0	94.10

Figures in parenthesis against recipient groups indicate sensitizing dose of larvae to respective donor groups. The data were submitted to χ^2 test with a 2×2 contingency table (with 1° of freedom). (Lewis, 1966). The tabulated value of χ^2 for 1° of freedom and at 5% level is 3.841, a value greater than this was considered significant. This statistical analysis clearly shows that sensitized cells from thymus and bone marrow are capable of transferring immunity.

30×10^4 thymus cells from donor groups A and B were injected i.p. into each mouse of the recipient groups a_1 and b_1 (each having 24 mice of same age and weight) respectively. Similarly recipient groups a_2 and b_2 also with 24 mice each received 50×10^4 bone marrow cells from the same donor groups. Control recipient groups c_1 and c_2 received thymus (30×10^4) and bone marrow (50×10^4) cells respectively from uninfected donor group C mice.

Each recipient mouse of all 6 groups was orally challenged with a single dose of 500 *A. caninum* larvae 7 days after cell transfer. Necropsies were made from different organs and muscles of mice initially at 6-h intervals following Baermann's technique after 3 h digestion in artificial gastric juice. The number of actively motile larvae recovered at each necropsy was recorded and results with their statistical analysis are presented in the table.

Result and discussion. Significant expulsion of the larvae took place in recipient groups a_1 (48.0%) and a_2 (47.3%) which received cells from donors sensitized with 1000 larvae in comparison to groups b_1 (31.4%) and b_2 (39.5%) which received cells from donors sensitized with 500 larvae at 6 h after challenge. Thereafter the percentage of larvae expelled increased rapidly with time. In groups a_1 and a_2 rate of decline in the number of larvae recovered was rather rapid, resulting in the total expulsion and/or destruction of larvae by 144 h (6th day) and 120 h (5th day) respectively. Recipients injected with cells from donors (group B) were more competent in expelling larvae from a challenge dose than the recipients which received unsensitized cells from control donors (group C). These results are statistically significant.

The results are surprising as they indicate clearly that sensitized thymus and bone marrow cells are able to confer immunity passively in *A. caninum* mouse model. These cells seem to play their role in CMI similar to that of mesenteric lymph node and peritoneal exudate cells in this model[17,18]. The expulsion of larvae from the gastrointestinal tract (which took place at 48 h after challenge) was found to be due to severe inflammation and to infiltration of sensitized cells. The release of histamine[21] furthermore adds to the conditions unfavourable for the maintenance of the larvae. Lung migration in the present work was very marked [17,18]. This may account for the slow destruction of larvae in muscles[22] in this series of experiments, in contrast to the results of Vardhani and Johri[18] who observed the presence of larvae in muscles at 4 h after challenge. The larvae did not appear in muscles in this case until upto 24 h after challenge and, thereafter, they persisted in the muscles for a considerably long time (upto zero recovery) although they were not so active.

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Immunological observations following vasectomy

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Summary. Lymphocytotoxic antibodies (LCA) against panels of normal lymphocytes and leukemic B-cells were demonstrated in vasectomized men. Since vasectomy is known to induce antibody formation to spermatozoa, the demonstration of these lymphocytotoxic antibodies may be related to antigenic constituents of spermatozoa such as HLA or B-cell alloantigens. Long term follow-up is needed to clarify the clinical significance of these antibodies.

In a previous study, 9 of 12 vasectomized men developed lymphocytotoxic antibodies (LCA) which were considered to be specific for, or at least cross-reactive with HLA antigens[2]. The present report of 10 additional subjects is based on a more extensive evaluation of LCA as tested not only against normal lymphocytes but also against leukemic B-cells. The occurrence of autoantibodies was also investigated.

Materials and methods. The study group comprised 10 men, aged 30–48 years, who had bilateral vasectomy under general anesthesia. Blood samples were drawn prior to vasectomy and at selected intervals following vasectomy for a period of up to 24 months. Blood samples were similarly drawn from age-matched controls comprising male patients who underwent minor surgical procedures and healthy male employees of the hospital. None of the 10 study cases nor any of the controls received blood transfusions prior to or during the course of study.

Lymphocytotoxic antibodies: Sera of the vasectomized men and of the control subjects were tested for LCA using a

Table 1. Lymphocytotoxic antibodies in vasectomized and control cases*

Group	Positive reactions/cases tested					
	Cases	Before surgery	After operation (months)			
			1–3	4–6	7–9	10–24
Vasectomy a	5	0	3.5	3.0	2.0	0.25
Vasectomy b	5	0.25–0.5	1.25	1.6	0	0.25
Surgical controls	7	0	0	0		
Non-surgical controls	10	0	0	0		

*LCA against panel of normal lymphocytes from 40 normal donors.