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Catecholamines in Organs of Immunosympathectomized Mice.* (30422)

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Levi-Montalcini and Angeletti(1) have reported upon the norepinephrine content of several organs in immunosympathectomized mice. Berk *et al*(2) have studied both the norepinephrine and epinephrine contents of a few organs in comparably treated rats. In preparation for studies on the physiology of the immunosympathectomized animals we have analyzed a number of organs in immunosympathectomized and control mice for both of the above catecholamines. The data to be reported here confirm and extend the observations noted above.

Methods. The sympathetic nerve growth promoting factor was isolated from mouse submaxillary glands, purified, and its activity assayed by chick embryo dorsal root ganglia. Antiserum was prepared from rabbit immunized with this factor and assayed for potency by inhibition of the growth factor on chick embryo ganglia. All these procedures were adapted from Cohen(3) and Levi-Montalcini and Angeletti(4). Newborn C₃H strain male mice were injected with the antiserum daily for one week, about 0.05 ml serum (titer about 1:1000 by the inhibition test *in vitro*) per gram body weight per day. A group of similar newborn control mice were injected with normal saline in the same manner. The mice were housed in an air-conditioned room and cared for according to our standard methods. When the animals were 4 weeks old, various organs were assayed for

epinephrine and norepinephrine content. The effectiveness of the immunosympathectomy of the experimental mice was checked by examination of the sympathetic chain of ganglia, and the cervical ganglia were studied histologically. The extensive reduction of the neurones in the experimental group was verified.

The animals, 13 (11-16) g in weight, were sacrificed by cervical dislocation. The hearts, brain, adrenal glands, spleen and kidney were removed immediately, fat and connective tissue trimmed away, blood washed off with a small amount of redistilled water, and blotted with filter paper. The tissues were then minced with scissors and transferred to a pre-weighed flask with a measured amount of 10% trichloroacetic acid. Organs from 8 to 20 mice were pooled as a single sample. The average weights of the organs from individual mice were: heart 58 (48-70) mg, brain 320 (290-380) mg, spleen 35 (28-45) mg, and kidney 45 (38-52) mg. The adrenal glands were not weighed. The preparation of tissue homogenates and the methods of determination of norepinephrine and epinephrine were essentially the same as those described in a previous paper from this laboratory(5).

Results. As shown in Table I, the catecholamine content of the heart, spleen, kidney, and especially the brain of the normal mouse is mainly norepinephrine, while the adrenal glands contain both epinephrine and norepinephrine, the level of the former being higher than that of the latter. In the im-

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TABLE I. Catecholamine Content in Various Organs of the Immunosympathectomized Mice.

Organs	Control			Experimental		
	No. of mice	Epinephrine	Norepinephrine	No. of mice	Epinephrine	Norepinephrine
		$\mu\text{g/g wet tissue}$			$\mu\text{g/g wet tissue}$	
Hearts (untreated)	9	.03	.40	12	.16	.08
	10	.00	.58	11	.00	.00
	10	.01	.69	8	.04	.00
				8	.15	.11
	(saline inj)	10	.06	10	.04	.08
	9	.12	.54	10	.02	.06
	9	.06	.69			
	Avg	.05	.56	Avg	.07	.06
Spleen (untreated)	10	.00	.43	12	.11	.05
	18	.05	.46	11	.06	.00
				16	.04	.02
	(saline inj)	10	.00			
	9	.01	.42	Avg	.07	.02
	9	.21	.26			
	Avg	.05	.38			
Kidney (untreated)	9	.00	.21	12	.05	.03
	20	.00	.21	11	.05	.00
	8	.00		16	.02	.00
				10	.05	.02
	(saline inj)	10	.00	10	.09	.05
	9	.01	.12			
	9	.00	.13	Avg	.05	.02
	Avg	.00	.16			
Brain (untreated)	9	.00	.38	12	.00	.32
	10	.00	.45	11	.02	.34
	10	.00	.36	8	.03	.46
	8	.00	.42	8	.00	.24
				10	.00	.44
	(saline inj)	10	.00	10	.00	.43
	9	.00	.36			
	9	.04	.37	Avg	.01	.37
	Avg	.00	.39			
Adrenal glands (untreated)		$\mu\text{g/pair glands}$			$\mu\text{g/pair glands}$	
	19	3.13	1.09	12	2.70	1.68
	18	3.66	.87	11	2.71	2.47
				8	2.20	1.79
	(saline inj)	10	2.44	8	2.70	1.65
	9	2.94	1.43	10	3.22	2.01
				10	3.33	1.70
	Avg	3.05	1.26			
				Avg	2.81	2.05

munosympathectomized group the norepinephrine content was markedly reduced in the heart, spleen and kidney, the percentages of control being 10.6, 5.3 and 12.5 for the heart, spleen, and kidney, respectively. The norepinephrine content of the brain was practically identical among the sample groups. There was a 60% increase in the mean norepinephrine content of the adrenal glands of the experimental over the control group which is statistically significant, but the small sample size should be kept in mind. There

was no overlap of values in the 2 series, although one value in the experimental series was identical with that in a control.

The epinephrine content of all the organs except the adrenal in both control and experimental groups was low, as expected, and was not significantly different in the two groups. The finding of trace amounts of epinephrine in the kidneys of sympathectomized animals is suggestive, but not conclusive, evidence of an alteration from the control because of limitations in accuracy of analysis

of such small quantities.

Discussion. The studies of Zaimis(6) have indicated that the immunosympathectomized animal shows a sensitization to both epinephrine and norepinephrine, comparable to the effect of surgical denervation. Brody(7) has also found increased sensitivity to norepinephrine and in addition noted that urinary excretion of catecholamine was depressed in immunosympathectomized rats. This preparation appears to be well suited for studies of performance of various organs deprived of sympathetic innervation.

The failure of the brain and the adrenal glands to show any decline in norepinephrine content after administration of the antiserum to the sympathetic nerve growth promoting factor might suggest that the catecholamine-producing cells in those organs react either quantitatively or qualitatively differently from peripheral sympathetic neurones. Berk *et al*(2) have reported that under antiserum treatment conditions which lead to virtual disappearance of sympathetic ganglion cells

the adrenal medullary cells remained intact.

Summary. In immunosympathectomized mice at one month after specific antiserum treatment there is almost complete (87-95%) disappearance of norepinephrine in heart, spleen and kidney. There is no change in brain content, but there is a probably significant increase in adrenal norepinephrine content. Values for epinephrine after immunosympathectomy are not significantly altered from control levels, except perhaps in the kidney.

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Interferon Production in Powassan Virus Infections of Chickens. (30423)

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Interferon appears rapidly in the serum of several experimental hosts following intravenous inoculation of large doses of certain viruses, bacteria and bacterial endotoxins(1,2,3). In mice, Baron *et al*(4) found that interferon was not detectable during later stages of nonfatal infection with influenza and lactic dehydrogenase viruses despite continued viral multiplication. However, a close relationship between interferon and virus titers persisted throughout infection with encephalomyocarditis virus. The role of interferon in recovery from viral infections has recently been reviewed(5,6,7).

Powassan virus(8), a group B arbovirus transmitted by ticks(9), produced inapparent

infections in young chicks inoculated subcutaneously with one to 100 mouse LD₅₀ of virus(10). Viremia reached maximum titers on the third or fourth day, falling rapidly thereafter. The present study reports the production of interferon following intravenous (IV) inoculation of massive doses of Powassan virus into chickens aged 6 days and 3 months.

Materials and methods. Tissue cultures. Primary cultures of chick embryo fibroblasts (CEF) were prepared by trypsinization of 11-day-old embryos from which the beak, eyes and limbs had been removed. The growth medium was a modified Gey's balanced salt solution(11) containing 0.25% lactalbumin hydrolysate and 0.1% yeast extract (GLY) together with 5% bovine serum

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