Influence of Hyper and Hypothyroidism on Susceptibility of Mice to Infection with Lansing Poliomyelitis Virus.* (20088)

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In seeking an explanation for the seasonal variation in incidence of poliomyelitis and for the predilection of the disease for the lower age groups certain investigators have questioned the role of the thyroid gland. Clinicians have reported that chilling seems to be a predisposing factor in some cases of poliomyelitis. The experimental test of this clinical observation was provided by Milzer *et al.*(1). These workers found that rhesus monkeys inoculated intracerebrally with Stimpert's BK strain of poliomyelitis virus and then subjected to chilling in cold water exhibited a higher incidence and greater severity of paralysis than did the controls. Holtman(2) studied the effect of environmental temperature on susceptibility of mice to poliomyelitis virus. He reported that the higher the acclimation temperature before inoculation, the shorter was the incubation period of the virus. The incubation period was lengthened somewhat when the mice were maintained following inoculation at temperatures lower than those of acclimation. Since these results appear to conflict with those of Milzer *et al.*, Holtman stated that the difference might be due to the suddenness of temperature change. Manifestly, also, the disease in mice differs from that in the monkey. Since it is well known that thyroxine secretion is increased upon exposure to cold, Holtman tested the effects of thiouracil and thyroactive substances upon susceptibility of mice to poliomyelitis(3). Mice receiving thiouracil invariably showed signs of paralysis and died earlier than the controls. Administration of thyroid extract or thyroprotein produced incubation periods longer than those in the controls or the hypothyroid mice. Gollan(4) could demonstrate no differences in susceptibility of mice injected with thyroxine to MM virus. The experiments reported here were carried out in an attempt to clarify these controversial findings and to determine the influence of hyper- and hypothyroidism upon susceptibility of mice to the Lansing strain of poliomyelitis virus.

Materials and methods. Mice: Webster Swiss mice from our own colony were used in all experiments. Handling and care of the animals have been described previously(5). The average age of the mice in series 145 and 148 at the time of inoculation was 32.4 days, and in series 146 was 37.6 days. Diets: The basal or control ration referred to as "optimum" had the following percentage composition: vitamin-free casein 19, sucrose 71.2, salts IV 4, B vitamin mix 0.5, choline chloride 0.3, corn oil§ 5. The 0.5 g of B vitamin mix contained the following in sucrose: thiamine hydrochloride 0.3 mg, riboflavin 0.3 mg, pyridoxine hydrochloride 0.3 mg, nicotinic acid 1.5 mg, calcium pantothenate 2 mg,

TABLE I. Dietary Regimens.

Diet No.	Type and composition of diet
1	Optimum (control)
2	Hyper*—Opt + .25% IC 16 days, then .1% IC
3	Hyper —Opt + .1% IC 14 days, then .05% IC
4	Hyper —Opt + .06% IC
5	Hyper —Opt + .08% IC
6	Hypo —Opt + .1% PTU 7 days, then .02% PTU
7	"Hyper-Hypo"—Opt + .1% IC and .1% PTU 7 days, then PTU .02%

* Hyper = Hyperthyroid; Hypo = Hypothyroid; Opt = Optimum.

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§ Mazola, fortified with oleum percomorphum (Mead Johnson and Co., Evansville, Ind.) to supply *ca.* 1800 I.U. vit. A and 260 I.U. vit. D per 100 g ration.

TABLE II. Summary of Fatalities and Virus Infection.

Series No.	Virus dilution	Group No.*	Ration No.	No. of mice	Fatalities,† %	Virus infection,‡ %	Total fatalities, %	AIP,§ days	AST,‖ days
145	10^{-2}	1U	1	7	0	—	0	—	—
		1I	1	28	11	89	100	7.6	8.3
		2U	2	7	29	—	29	—	—
		2I	2	27	48	52	100	7.2	6.8
		3U	6	6	0	—	0	—	—
		3I	6	28	0	96	96	5.9	7.4
		4U	7	7	0	—	0	—	—
		4I	7	25	52	48	100	6.3	6.9
146	10^{-2}	1U	1	7	0	—	0	—	—
		1I	1	40	8	88	96	7.8	9.6
		2U	3	7	29	—	29	—	—
		2I	3	29	34	62	96	7.9	8.0
		3U	6	7	0	—	0	—	—
		3I	6	40	10	88	98	7.8	9.6
148	10^{-2}	1U	1	7	0	—	0	—	—
		1I	1	34	3	85	88	6.8	7.9
		2U	6	7	0	—	0	—	—
		2I	6	33	9	88	97	7.3	8.7
		3U	4	7	14	—	14	—	—
		3I	4	20	50	50	100	5.7	7.1
		4U	5	7	43	—	43	—	—
		4I	5	21	38	57	95	7.8	8.3
148	10^{-3}	1U	1	7	0	—	0	—	—
		1I	1	14	14	64	78	8.3	9.6
		2U	6	7	0	—	0	—	—
		2I	6	14	14	57	71	9.1	9.1

* U = uninoculated controls; I = inoculated. † Without signs of infection.
‡ Showed paralysis before death. § Avg incubation period in mice showing paralysis.
‖ Avg survival time of mice dying with or without signs of paralysis.

i-inositol 100 mg, sodium p-aminobenzoate 100 mg, biotin 10 micrograms, folic acid 25 micrograms. To provide bulk and reduce the hygroscopicity of the ration 10 g of Cellu flour were added per 100 g of diet. Iodinated casein (IC)‖ and 6-n-propyl-2-thiouracil (PTU)¶ were used to produce the hypothyroid and hypothyroid states, respectively. These substances were added to the diet at the expense of sucrose in amounts established by Miller and Baumann(6) for the rat. The compositions of the various diets are given in Table I. The "hyper-hypo" diet (7) contained both IC and PTU in a combination which produces only a slightly elevated BMR in the rat. In the hyperthyroid diets containing IC the amounts of thiamine, riboflavin, pyridoxine, biotin and folic acid were doubled and 7 μg vitamin B_{12} per 100 g of ration were included. Rations and water were fed *ad libitum*. *Virus.* In series 145 and 148 the mice received their respective rations for 7 days before inoculation and in series 146 for 11 days. In series 145 and 146 the inoculum was 0.03 ml of a 10^{-2} brain-cord suspension of Lansing virus, containing 100 LD50, administered intracerebrally. In series 148 dilutions of 10^{-2} and 10^{-3} were employed. All animals were observed daily, and the controls were weighed twice weekly.

Results and discussion. In Series 145 all of the hypothyroid mice (Group 3I, Table II) showed paralysis before death. The disease also appeared to be more fulminating in the hypothyroid animals than in the controls since incidence of paralysis and death of the former group exceeded that of the latter during the first 6 days following inoculation. The average incubation period (AIP) in the hypothyroid mice was somewhat less than in the controls. In the hyperthyroid group (2I) there was a high incidence of deaths without

‖ Protamone; Cerophyl Laboratories, Kansas City, Mo.

¶ Furnished by Calco Chemical Division, American Cyanamid Co., Bound Brook, N. J.

previous signs of paralysis. Many of these deaths were undoubtedly due to thyrotoxicosis since 2 of the 7 uninoculated control animals died. However, it is of interest that group 4I, which received IC and PTU in a ratio which maintains the BMR of rats only slightly above normal, manifested almost identical incidences of paralysis and fatalities without signs of paralysis as did the hyperthyroid group. It is also noteworthy that none of the uninoculated controls for this group died. The differences in AIP and AST between the various groups were not great, although it is evident that these figures for the normal group were greater than for any of the other groups, and that the shortest AIP was in the hypothyroid group. In series 146 the amount of IC in the diet of the hyperthyroid mice was reduced, but still 2 of the 7 uninoculated controls died. Again there was a high incidence of deaths without signs of paralysis in the inoculated group (2I). In this series the hypothyroid group (3I) did not differ from the normal group (1I).

The mice selected for series 148 were of the same age as those used in series 145, and a further virus dilution of 1:1000 was employed with normal and hypothyroid animals. There were no differences between normal (1I) and hypothyroid (2I) mice at either virus dilution with the exception of a slightly prolonged AIP in the latter. In both of the hyperthyroid groups (3I and 4I), which received less IC than was fed in the other 2 series, the incidence of fatalities without signs of paralysis was high. In group 3U only 1 of the 7 controls died, whereas in the inoculated group (3I) 50% died without signs of paralysis. This seems to bear out the results obtained in series 145, namely that even a slightly hyperthyroid condition in mice either favors the proliferation of virus or imposes sufficient stress on the already taxed metabolism to cause death in a large percentage of the animals before signs of paralysis are evident.

The results represented here are at variance with those obtained by Holtman, although in series 145 the hypothyroid condition did seem to favor slightly the progress of the disease, further experiments failed to substantiate this. Furthermore, the administration of thyroprotein did not increase the incubation period, but it did bring about a high incidence of deaths without signs of paralysis. It may well be that the virus employed by Holtman was not affected in the same way by the BMR of the host as is the Lansing strain of poliomyelitis. It is also possible that as Holtman has suggested there is a narrow critical range within which variation in the metabolic rate may alter susceptibility to poliomyelitis. More precise experiments should determine if such a critical range actually exists.

Summary. The influence of hyper- and hypothyroidism upon susceptibility of mice to infection with Lansing poliomyelitis virus has been studied. In one series hypothyroidism seemed to favor a higher incidence of paralysis, but this was not substantiated in two other series. The only consistent deviation from the normal course of infection was an increased incidence of deaths without previous signs of paralysis in hyperthyroid mice.

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