Endocrine Factors in Pathogenesis of Experimental Poliomyelitis in Hamsters.* Role of Inoculatory and Environmental Stress. (22206)

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Golden hamsters are susceptible to intracerebral but refractory to intraperitoneal inoculation of MEF_1 virus(1,3). Susceptibility of intracerebrally inoculated animals undergo significant alterations as a result of natural or experimental disturbances in the adrenal-testes endocrine equilibrium(2). This paper reports the effect of interacting stress, endocrine function, and route of inoculation upon poliomyelitis susceptibility in hamsters.

Materials and methods. Male Golden hamsters 25-30 g were inoculated with MEF_1 virus or control sterile mouse brain emulsion (SMBE) in equal volume. Animals were killed in groups of 20, daily for 5 days, then at 7, 10, 12, 15, and 28 days following inoculation. Weights of adrenal glands, testes, thymus, and interscapular brown fat (ISB) of animals inoculated with virus were compared with those inoculated with the SMBE and with uninoculated controls sacrificed the same days. The following volumes of MEF_1 virus suspension, diluted 1/100 in saline, were used: 0.05 ml intracerebrally, 0.02 ml intraspinally, 0.5 ml intraperitoneally, and 0.2 ml intramuscularly and subcutaneously. Paralytic incidence and mortality were noted daily for 21 days. Animals dying without paralytic symptoms have been excluded. Histological examination of the spinal cord, and mouse inoculation were periodically used to control clinical observations. The experiments were repeated a number of times and the results were averaged per group, per season, and per year. Except for the investigation of climatic stress, all animals were kept in the constant environment of air conditioned rooms at approximately 23°C. The results were analyzed by statistical procedures previously described(2). The association between series, *i.e.*, proportion

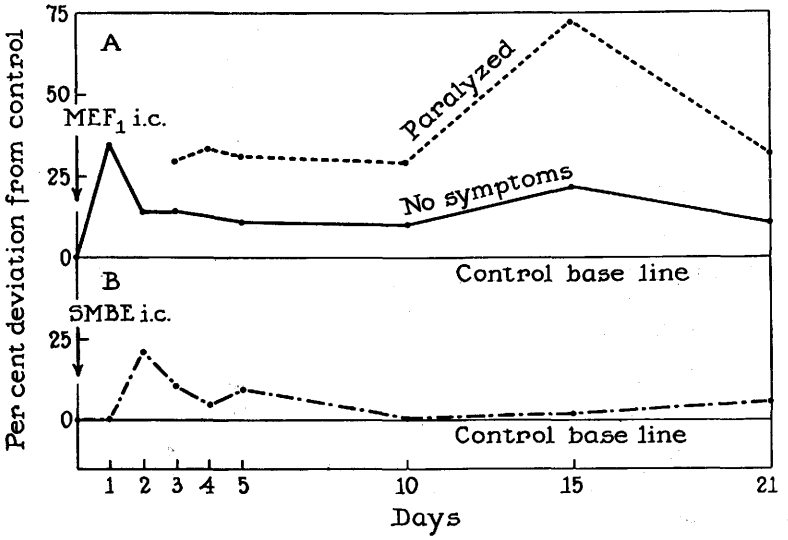

FIG. 1. Percent deviation from control of mean adrenal weight following intracerebral injection in Golden hamsters: A. in 206 animals injected with MEF_1 virus; B. in 625 animals injected with sterile emulsion (SMBE). Base line represents non-injected controls sacrificed the same time and in equal number as experimental animals.

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TABLE I. Effect of Preparatory Pathogen-Free Intracerebral Injection (SMBE) upon Susceptibility of Golden Hamsters to Poliomyelitis Virus (MEF_1) Inoculated Intraperitoneally.

Treatment		No. animals	Incidence of paralysis, %	Statistical significance "P"
Intracerebral	Intraperitoneal			
Sterile M.B.E.* .05 cc	MEF_1 virus 5 × .5 cc	125	16.00	.0001
None	*Idem*	254	3.93	

* M.B.E. = Mouse brain emulsion.

of poliomyelitis incidence (Y axis) and proportion of hypertrophic adrenals (X axis), under varying stressing conditions, was analyzed by using the correlation technic(18,19).

Results. Effects of inoculatory trauma. The effect of intracerebral inoculation upon the endocrine glands was studied on 1500 hamsters. Animals inoculated with virus intracerebrally averaged significant adrenal hypertrophy within a 5-day period following injection (Fig. 1). Alterations of the average adrenal weight noted beyond this period were statistically insignificant. Average weights of thymus, testes, and ISB showed no significant changes within the initial 5-day period. Endocrine alterations of the same pattern were found when SMBE was substituted for the viral inoculum (Fig. 1), indicating that the observed adrenal hypertrophy was produced by the intracerebral inoculation, rather than by MEF_1 virus. Among animals inoculated with virus, adrenal hypertrophy was found in the paralyzed animals regardless of elapsed time between intracerebral inoculation and start of paralysis (Fig. 1). To determine whether the refractory state to intraperitoneal inoculation virus could be eliminated by a preliminary sterile intracerebral injection, animals were injected intracerebrally with SMBE, after which MEF_1 virus was given intraperitoneally every other day, 3 or 5 times. Poliomyelitis, confirmed clinically and pathologically, developed in 12-25% of the treated animals. Control groups, repeatedly inoculated with virus intraperitoneally, only developed the disease in 2-6% (Table I).

Persistence of MEF_1 virus in various inoculated tissues. A single intraperitoneal inoculation of virus given the same day as the sterile intracerebral injection did not produce poliomyelitis. This suggests that rapid disappearance of the virus precluded dissemination before initiation of the endocrine alterations which usually appear 48 hours after the intracerebral trauma (Fig. 1). To determine whether there is any significant difference in persistence of poliomyelitis virus within the cranial and peritoneal cavities, several groups of infected animals were given a single injection of 5 mg cortisone at different intervals following virus inoculation. Cortisone produced

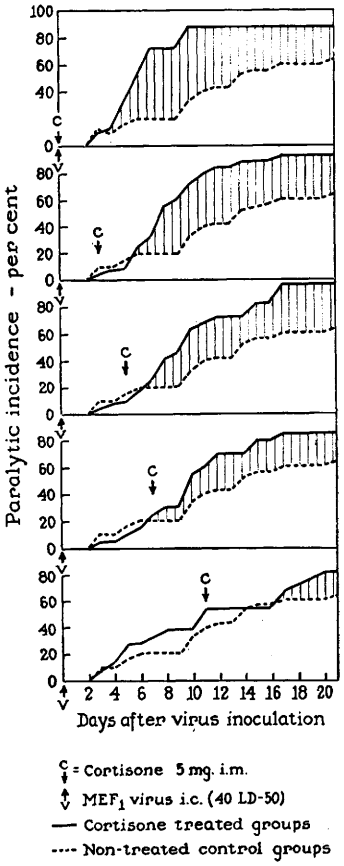

FIG. 2. Conversion of quiescent poliomyelitis into paralytic disease with the aid of cortisone given at varying intervals after intracerebral inoculation of virus.

TABLE II. Frequency of Hypertrophic Adrenals According to Age in Populations of Normal Hamsters Kept in Constant Environment of An Air Conditioned Laboratory.

Body wt, g	No. animals	Proportion of adrenals over 14 mg/100 g, %
25–35	403	17.61
35–50	538	19.70
50	135	46.66

enhancement of paralytic incidence as late as 11 days following intracerebral inoculation, indicating that potent virus was still present in a quiescent state. Conversely, no paralysis or death was produced by cortisone beyond the third day following intraperitoneal, intramuscular or subcutaneous inoculation of virus thereby suggesting that the virus was rapidly destroyed when introduced peripherally, unless endocrine alterations like those induced by a sterile intracerebral injection favor its progression and dissemination.

Endocrine reactivity to stress and susceptibility to poliomyelitis virus. Assuming that individual or seasonal variations in susceptibility to poliomyelitis may be associated with individual or seasonal deviations from the general pattern of endocrine reactivity to inoculatory trauma, adrenal reactivity was investigated and compared with susceptibility to poliomyelitis virus in 5000 hamsters maintained in the relatively constant environment of an air-conditioned laboratory. The weight of 14 mg per 100 g body was considered as the boundary limit between normal and hypertrophic adrenals. This represents the median figure between the mean value found in 299 hamsters submitted to normal climatic fluctuations and the mean adrenal weight of 486 animals kept in air-conditioned rooms and sacrificed during the same seasonal periods as the former. Actually, more than 80% of 941 young hamsters (25-50 g), kept in a constant environment and autopsied at various periods of 1954, showed adrenals weighing less than 14 mg/100 g. The frequency of adrenal hypertrophy increased with the age, due possibly to repeated occasional stresses associated with daily care, *i.e.*, feeding, cleaning, etc. (Table II).

When SMBE was injected intracerebrally in young hamsters, 33% of 327 animals sacrificed during the following 5 days, showed adrenal hypertrophy. When poliomyelitis virus was similarly injected in 372 animals, 35% developed paralytic symptoms during the first week of observation. Table III shows that seasonal variations observed in adrenal reactivity to inoculatory trauma were paralleled by similar fluctuations in the paralytic incidence of the first week following inoculation.

As may be seen from Table III, the paralytic incidence of the first week following intracerebral inoculation of virus, represents only 2/3 of the total incidence recorded during a standard observation period of 21 days under constant environmental conditions. Based upon the assumption that the additional 1/3 paralytic incidence may be incidental to additional stresses, such as daily examination of the animals, 100 hamsters were inoculated with MEF_1 virus intracerebrally then separated into 2 groups. One group was examined routinely every day, while the other group was kept undisturbed for 21 days. Table IV shows that both morbidity and mortality were higher in the group examined daily, than in the non-disturbed group.

TABLE III. Seasonal Fluctuations in Adrenal Reactivity to Inoculatory Trauma as Compared with Parallel Variations in Susceptibility to Poliomyelitis Virus.

	Intracer. inj. S.M.B.E.*		Intracer. inj. of MEF_1 virus		
Seasonal period	No. animals	Incidence of adrenal hypertrophy, %	No. animals	Incidence of paralysis 1st wk, %	Total paralytic incidence 3 wk, %
5/54	99	31	89	38	61
9/	129	24	147	26	40
2/55	99	46	136	42	66
Yearly avg	327	33	372	35	55

* S.M.B.E. = Sterile mouse brain emulsion.

TABLE IV. Effect of Daily Examination upon Paralytic Incidence and upon Mortality from Poliomyelitis in Golden Hamsters Inoculated with MEF_1 Virus Intracerebrally, 50 Animals in Each Group.

Groups	Paralytic incidence, %	Mortality, %
Examined daily	64	44
Not disturbed	52	30

Though the difference was found statistically insignificant, it persisted when the experiment was repeated on 175 additional animals.

The role of environmental stress in enhancing paralytic incidence in intracerebrally inoculated hamsters is demonstrated in Table V. The paralytic incidence is significantly higher in animals kept in the variable environment of normally heated rooms than in animals kept in air-conditioned laboratories. A significant increase in belated paralytic incidence, *i.e.*, appearing beyond the first week following inoculation, seems to be the main effect of the stressing environment. This explains the paradoxical observation that, despite the greater morbidity, the mean incubation period was longer in a varying than in a constant environment due to the number of belated paralytic cases provoked by environmental stress (Table V).

Groups of animals maintained in refrigerated rooms at 5°C or dark rooms at 23°C showed adrenal hypertrophy in a percentage similar to the percentage of paralytic poliomyelitis observed under similar environmental conditions (Table VI).

Statistical analysis of the association between series in 7 pairs of groups totaling 1602 animals submitted to varying experimental stresses as shown in Tables III and VI, revealed a linear correlation of high statistical significance between the percentage of adrenal hypertrophy in one series and the percentage of paralytic poliomyelitis in the other. As a corollary observation, 82% of the animals developing poliomyelitis showed adrenals over 14 mg/100 g, at the time when the paralytic symptoms first became evident.

Comparative effects of various inoculation procedures. In hamsters kept in an air-conditioned environment, a sterile intraspinal in-

TABLE V. Progression of Paralytic Incidence following Intracerebral Inoculation of MEF_1 Virus in Groups of Hamsters Kept in Variable Environment as Compared to Groups Kept in Air Conditioned Rooms at 23°C.

Environment	No. animals	Early paralysis 1st wk, %	Significance "P" of difference	Late paralysis 2nd & 3rd wk, %	Significance "P" of difference	Total paralysis 3 wk, %	Significance "P" of difference	Mean incubation period, days
Variable (normally heated room)	600	26.16		35.50		61.66		9.40
Constant (air conditioned)	1333	33.53	.001	19.05	Highly significant*	52.58	.001	7.53

* .00000000001.

TABLE VI. Incidence of Adrenal Hypertrophy as Compared to Incidence of Paralytic Poliomyelitis in Groups of Hamsters Submitted to Various Environmental and Dietary Conditions for 3 Weeks.

Treatment	Adrenal reactivity			Susceptibility to MEF_1 intracer.	
	No. animals	Avg adrenal wt, mg/100 g	Incidence of adrenal hypertrophy, %	Total No. animals	Paralytic incidence, %
Refrigeration	139	15.14	61.39	86	65.11
Dark room	183	15.07	52.33	93	58.06
Protein enriched diet	125	14.17	54.40	132	59.84
Idem + S.M.B.E., intracer.*	144	14.76	52.77		

* Intracer. inoculation of .05 cc sterile mouse brain emulsion.

jection was followed by a larger proportion of adrenal reactions than an intracerebral one, *i.e.*, 46% as compared to 19% during April 1955. The incidence of paralytic poliomyelitis also was significantly higher in intraspinally than in intracerebrally inoculated animals. The mean incubation period was shorter after intraspinal than after intracerebral inoculation of virus (Table VII). An intraspinal injection with SMBE prior to and on the same day as the intracerebral inoculation of MEF_1 virus, raised the incidence of paralysis to the same level as in animals receiving virus intraspinally. The mean incubation period remained however, within the range of intracerebrally inoculated animals. A sterile intracerebral injection preceding administration of virus intraspinally did not change significantly the paralytic incidence or the incubation period as compared to controls inoculated only intraspinally (Table VII).

Adaptation to stress and susceptibility to poliomyelitis virus. Intracerebral inoculation of MEF_1 virus after varying periods spent in cold environment, resulted in a decreased incidence of poliomyelitis in proportion to the time spent in the cold environment prior to inoculation, *i.e.*, 70% when refrigeration started on the day of inoculation, 66% in groups refrigerated for 5 days prior to inoculation, and 57% in groups refrigerated for 10 days preceding the inoculation. Chronic adrenal hypertrophy was found in 86% of hamsters kept under protracted climatic stress. When poliomyelitis virus was injected intracerebrally in similar groups, paralytic incidence was only 66%, suggesting that, with time, adaptation had compensated for the enhancing effect of the adrenal hyperfunction in about 20% of these continuously stressed animals (Table VIII). Conversely, spontaneous adrenal hypertrophy was found in less than 20% of the animals in the air-conditioned environment, while paralytic incidence that followed intracerebral inoculation of virus exceeded 50% (Table VIII). As shown above, in such constant environment, 2/3 of the total paralytic incidence depends upon adrenal reactions induced by inoculatory trauma which takes over the role of the eliminated environmental stress.

In a stress environment, the enhancement of viral progression by inoculatory trauma seems to be reduced, as suggested by the fact that the incidence of early paralysis was significantly lower in an inconstant, than in air-

TABLE VII. Effect of Inoculatory Trauma upon Paralytic Incidence and upon Mean Incubation Period in Hamsters Receiving MEF_1 Virus Either Intracerebrally or Intraspinally.

Inoculation		No. animals	Total paralytic incidence, %	Early paralysis 1st wk, %	Late paralysis 2nd & 3rd wk, %	Mean incubation period, days
Intracer.	Intraspinal					
MEF_1 virus	None	82	39.02	28.04	10.97	6.50
None	MEF_1 virus	77	68.83	68.83	0	3.33
MEF_1 virus	S.M.B.E.*	78	66.66	48.71	17.94	6.98
S.M.B.E.	MEF_1 virus	77	62.33	55.84	6.49	4.45

* S.M.B.E. = Sterile mouse brain emulsion.

TABLE VIII. Effect of Normal Climatic Fluctuations upon Adrenal Weight, Susceptibility to Poliomyelitis Virus and Mean Incubation Period in Hamsters as Compared to Controls Maintained in a Constant Environment.

		Spontaneous adrenal hypertrophy			Susceptibility to MEF_1, intracer.			
Period of inoculation	Environmental conditions	No. animals	Average adrenal wt, mg/100 g	Proportion adrenals over 14 mg /100 g, %	No. animals	Paralytic incidence, %	Mortality, %	Mean incubation period, days
4/ 1/53-5/15	Variable*	60	17.09	86.66	139	66.90	38.84	9.39
4/ 8/54-5/14	Constant†	58	12.16	20.68	73	61.64	36.98	5.76
9/ 1/53-10/30	Variable	179	17.17	78.20	218	65.13	36.69	8.80
9/13/54-10/19	Constant	156	11.72	20.50	158	49.36	20.25	5.97
11/13/53-11/18	Variable	32	15.70	53.12	86	61.62	27.90	10.70
11/ 5/54-11/19	Constant	95	11.87	9.46	128	59.37	23.43	7.98

* A normally heated room. † Air conditioned at 23°C.

conditioned environment, despite a total paralytic incidence significantly higher in the former than in the latter (Table V). The paradoxical reduction of early paralytic cases in the fluctuating environment might have resulted from a reduction in the number of adrenal responses to inoculatory trauma, due either to temporary exhaustion of reactivity or to adaptation caused by the repeated environmental stress preceding the inoculation.

Effect of diets. A diet enriched with 1% casein hydrolysate, in addition to the normal Rockland mouse diet, increased the incidence of adrenal hypertrophy from 10% in the controls to 50% suggesting either a direct effect of the diet, or an enhancement of the adrenal reactivity to minor environmental stresses (20). The same diet enhanced significantly the paralytic rate following intracerebral inoculation of virus. As in the case with environmental stress, the casein enriched diet increased the incidence of belated paralysis to 27% from 16.76% in controls and also slightly increased the mean incubation period.

Discussion. The above findings emphasize the importance of 4 factors in the pathogenesis of experimental poliomyelitis: (a) inoculatory or environmental stress, (b) endocrine reactivity to stress, (c) persistence of inoculated virus, and (d) correct experimental timing. The effect of stress depends upon endocrine reactivity. Increased reactivity due to seasonal or dietary conditions enhances, while adaptation or temporary exhaustion of endocrine reactivity diminishes the effect of stress upon susceptibility to poliomyelitis. On the other hand, due to compensatory phases in the evolution of an endocrine disturbance, susceptibility varies with the timing of inoculation, in relation to the endocrine status present at time of viral inoculation (2). Some effects of cortisone in poliomyelitis infected hamsters give suggestive information regarding the mechanism by which the adreno-cortical hyperfunction induced by stress, may influence viral progression. Thus extensive spread and multiplication of virus in certain tissues is associated with the suppression of the non-specific inflammatory barrier produced by cortisone (1,4-9,11). Cortisone also favors accelerated and protracted viral multiplication in the brown fat cells in association with alterations in lipid metabolism(3,10,11). Experiments in progress demonstrate that stress enhances significantly the multiplication and persistence of MEF_1 virus in the brown fat of hamsters and also has a belated cortisone-like effect upon lipid metabolism. This effect upon persistence of virus may partly account for the high proportion of belated paralytic cases observed in a stress environment.

Cortisone induces viremia in hamsters by enhancing extraneural multiplication of virus, by suppressing inflammatory barriers, and by interfering with early antibody formation (1,8,9). According to Bodian, the viremia level is a main determinant of spinal cord invasion(12,13), and thus may account for the high paralytic rate observed in cortisone-treated animals.

Stress is also able to provoke viral invasion of the spinal cord. Thus, it has been shown that the paralytic incidence is significantly increased by a preliminary sterile intraspinal injection in intracerebrally inoculated, and by a preliminary sterile intracerebral injection in peripherally inoculated hamsters. A pathogen-free intramuscular injection increases paralytic rate and favors localization of the paralysis to the injected extremity of monkeys(12,13), and humans(16,17). This provoking effect of stress seems to be mediated by the resultant circulatory alterations which increase the blood supply to the corresponding part of the spinal cord(14,15) so that circulating virus can more readily penetrate into that region and set up an initial infection therein(13).

From studies thus far carried out, it may be concluded that properties like pathogenicity, invasiveness, etc., that are usually attributed to virus may actually depend on temporary alterations induced in the physiology of the host by factors as common as nutrition and environmental adaptation.

Summary. 1. The incidence of paralytic poliomyelitis in experimentally infected hamsters has been found to depend upon a variety of factors such as inoculatory trauma, environmental stress, and diet. A linear correlation was found between incidence of adrenal hypertrophy and incidence of paralytic disease induced by the above factors in 2 series of hamsters inoculated simultaneously, one with pathogen-free material, the other with MEF_1 virus. 2. Seasonal fluctuations in adrenal reactivity to the inoculatory trauma were closely associated with parallel fluctuations in susceptibility to poliomyelitis. Either, adaptation to chronic environmental stress, or rapid disappearance of the virus from certain inoculated tissues, interfered with the above correlation. 3. The mechanism by which endocrine glands and hormones may influence the progression of virus is discussed.

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1. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 573.

2. Teodoru, C. V., and Shwartzman G., *J. Exp. Med.*, 1954, v100, 563.

3. Shwartzman, G., Aronson, S. M., Teodoru, C. V., Adler, M., and Jahiel, R., *Ann. N. Y. Acad. Sci.*, 1955, v61, 869.

4. Aronson, S. M., and Shwartzman, G., *Am. J. Path.*, 1953, v29, 381.

5. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

6. Howe, H. A., and Bodian, D., *Commonwealth Fund N. Y.*, 1942, v99.

7. Rivers, T. M., *Stanford University Press*, 1939, 42.

8. Shwartzman, G., and Aronson, S. M., *Ann. N. Y. Acad. Sci.*, 1953, v56, 793.

9. Shwartzman, G., *J. Mt. Sinai Hosp.*, 1954, v21.

10. Aronson, S. M., Teodoru, C. V., Adler, M., and Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1954, v86, 767.

11. Aronson, S. M., Shwartzman, G., and Teodoru, C. V., *J. Neuropath., & Exp. Neurol.*, 1955, v14, 109.

12. Bodian, D., *Am. J. Hyg.*, 1954, v60, 358.

13. ———, *Ann. N. Y. Acad. Sci.*, 1955, v61, 877.

14. Trueta, J., and Hodes, R., *Lancet*, 1954, v265, 908.

15. Levine, R., *Bull. Acad. Sci. Med.*, 1952, v8, 13.

16. Farmer, A. D., *M. J. Australia*, 1955, v1, 141.

17. Trueta, J., *Ann. N. Y. Acad. Sci.*, 1955, v61, 883.

18. Arkin, H., and Colton, R. R., *Statistical Methods*, Barnes & Noble, Inc., New York, 1953, 74.

19. Fisher, R. A., *Statistical Methods for Research Workers*, Hafner Publ. Co., New York, 1954, 206.

20. Selye, H., *J. Clin. Endocrinol.*, 1946, v6, 117.

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