agent responsible for the difference in insulin responses of these 2 experimental animals. The experiments employing a combined hormonal regimen therefore may provide a clue as to the balance prevailing between these 2 blood sugar-raising agents in the hormonal regulation of normal carbohydrate metabolism. It has been suggested that growth hormone, by interfering with peripheral utilization of sugar, raises the blood sugar and this eventually results in the exhaustion of the pancreas(3). To this relationship of growth hormone to the pancreas should be added an important interrelationship of growth hormone with the adrenal cortex on the basis of the above experiments.

Summary. 1. Continued administration of cortisone or hydrocortisone regimens, in dosages (0.83 to 1.2 mg kg day) sufficient to restore the carbohydrate metabolism of adrenalectomized dogs to normal, completely abolished the insulin hypersensitivity, secondary hypoglycemia of the glucose tolerance test, and adrenaline resistance of hypophysectomized dogs without the production of insulin resistance, diabetes, or abnormally high adrenaline responses. 2. Continued daily administration of a potent "diabetogenic" growth hormone (1.0 to 1.5 mg kg day) in conjunction with the cortisone or hydrocortisone regimens did not alter the carbohydrate metabolism of the hypophysectomized dogs previously observed on the steroid regimens alone, *i.e.*, it remained normal as judged by the above criteria. 3. The insulin resistance, diabetes, and toxic manifestations produced by growth hormone regimens alone were not observed during combined adrenocortical steroid-growth hormone regimens and thus, a more prolonged period of growth hormone administration was made possible. 4. It is suggested that a balance exists between growth hormone and the adrenocortical steroids in the regulation of the carbohydrate metabolism of the normal organism. This balance, in part, may express itself as an antagonism between the two either in the liberation of another factor or in the transformation of growth hormone.

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Attempts to Demonstrate Interference Between Coxsackie and Poliomyelitis Viruses in Mice and Monkeys. (19900)

N. F. STANLEY. (Introduced by G. Dalldorf.)

From the Institute of Epidemiology and Preventive Medicine, Prince Henry Hospital, Sydney, Australia.

Using infant mice Dalldorf(1) demonstrated interference between a group B strain of Coxsackie virus and the Lansing strain of poliomyelitis as evidenced by decreased frequency and prolongation of the course of poliomyelitis, and by survival. On the other hand, Sulkin and Manire(2) reported that the inoculation of Lansing poliomyelitis virus into infant mice offered some slight protection to infection with a group A Coxsackie virus.

No evidence of interference was obtained by Howitt and Nichols(3) when mixtures of Coxsackie and poliomyelitis viruses were given to rhesus monkeys. The present report describes the results obtained with locally and freshly isolated strains of poliomyelitis and Coxsackie viruses occurring in New South Wales during the 1950-51 epidemic of poliomyelitis (Stanley *et al.*)(4) and with the MEF1 strain of poliomyelitis.

Materials and methods. Virus strains. The MEF1 strain of poliomyelitis virus was obtained through the courtesy of the George Williams Hooper Foundation, University of California Medical Center, California. After 10 passages by the intracerebral route in adult mice, the strain was adapted to suckling mice by 7 successive passages by the intracerebral route. The Cobbett strain of poliomyelitis was used in the monkey experiments. This is a Brunhilde-like virus and its properties are described elsewhere (Stanley and Dorman) (5). The Coxsackie viruses used in these experiments comprised both group A (myositis producing) and group B (encephalitis and myositis producing) strains. All strains were isolated in Sydney during the 1950-51 epidemic of poliomyelitis. *Animal stock.* The mice used were bred in this laboratory's animal house and were of the same stock used for earlier investigations. The monkeys were imported from Singapore and consisted of *M. irus, M. nemestrinus,* and *M. mulatta.* All appear to be equally susceptible to infection with Australian strains of poliomyelitis.

Experimental. Experiments with mice. 1) *Effect of cerebral trauma on susceptibility of mice to infection with the MEF1 strain of poliomyelitis by the intravenous route.* Adult mice were inoculated by the intracerebral route with 0.03 ml sterile broth, or 0.03 ml of a 10% suspension of mouse tissue infected with Coxsackie virus. After intervals of 15 minutes, 60 minutes, and 6 hours, 0.01 ml of a suspension of MEF1 infected mouse cords was given by the intravenous route. Eight animals were used in each group, and they were observed for 3 weeks. No paralysis or death was observed, irrespective of whether broth or a Coxsackie virus suspension in broth was used to produce cerebral trauma.

TABLE I. Titration of MEF1 Strain of Poliomyelitis in Adult Mouse Brain in Presence of Group A (S4) and Group B (E46) Coxsackie Virus.

MEF1 virus: Dilution	In the presence of	Time in days to paralysis or death: 2	3	4	5	6	7	8	9	10	11	12	13	14	15	No. of mice paralyzed	Average incubation time, days	Average incubation time adjusted to a reading of 1 for control group
10^{-1}	Broth	—	3	1	—	—	2	1	—	—	—	—	1			8	6	1
	S4	—	3	2	1	—	—	2	—	—	—					8	4.7	.8
	E46	—	—	—	1	—	—	1	2	2	1	—	1			8	8.7	1.5
10^{-2}	Broth	—	—	1	—	—	—	3	2	2						8	8.3	1
	S4	—	3	1	1	—	1	2	—	2	1	2	—	—	1	8	5.1	.6
	E46	—	1	—	—	—	—	—	2	2						8	10	1.2
10^{-3}	Broth	—	—	2	1	1	—	1	2							8	7.1	1
	S4	2	—	3	—	1	1	1				—	1	1	1	8	4.6	.6
	E46	—	—	2	—	—	1	—	1	—	—	—	2	—	—	7	10.5	1.5
10^{-4}	Broth	—	—	—	—	—	—	—	1	1	1	1	—	1	—	6		
	S4	—	—	1	—	—	—	—	2	—	2	1	—	—	—	8		
	E46	—	—	2	—	—	1	—	—	—	1	1	—	—	—	5		
S4 alone		—	—	—	—	—	—	—	—	—	—	—	—	—	—	0		
E46 alone		—	—	—	—	—	—	—	—	—	—	—	—	—	—	0		

2) *Titration of the MEF1 strain of poliomyelitis (in the presence of Coxsackie viruses) in adult mouse brain.* A freshly prepared suspension of mouse cord infected with MEF1 virus was diluted 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} 1) in broth, 2) in a suspension of S4 Coxsackie virus in broth, and 3) in a suspension of E46 Coxsackie virus in broth. The S4 Coxsackie virus is a group A strain and the broth suspension used was prepared from infected mouse limbs and used at a dilution of 10^{-2}. The E46 strain is a group B virus and a 10% suspension of infected suckling mouse brain was used as a diluent in this case. Eight adult mice were each inoculated by the intracerebral route with 0.03 ml of each dilution. The mice were observed for 15 days. The results are recorded in Table I. The entire number of 32 mice inoculated with MEF1 + S4 dilutions became paralyzed or died in a shorter interval of time than the mice inoculated with the MEF1 + E46 mixture. In the latter group 28 of the mice developed paralysis. This is suggestive of slight enhancement with the group A strain. Differences between the 3 titrations are more clearly seen by reference to the last column in Table I. In this column the average incubation period has been worked out and adjusted so that the control group always gives a figure of unity. It is seen that with all dilutions of the MEF1 strain the incubation period for group B Coxsackie mice is approximately double that of the group A Coxsackie mice, and the figure for the control is somewhere in between. This would suggest that there is enhancement with group A Coxsackie virus and interference with group B virus.

It was thought that the time interval between the inoculation of the poliomyelitis and the Coxsackie viruses may have some bearing on the demonstration of the interference phenomenon. This was investigated by inoculating mice intracerebrally with Coxsackie virus strain E46, 48 hours before, and 24 hours after, the intracerebral inoculation of the MEF1 strain. No significant alteration in time of onset of paralysis or the number of mice paralyzed could be demonstrated.

One interesting feature of the investigations with the MEF1 strain of poliomyelitis was the

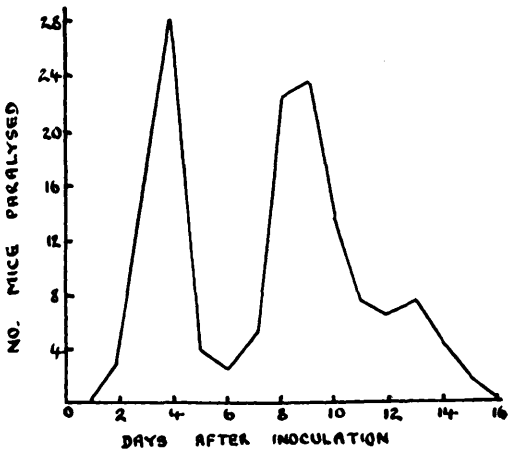

FIG. 1. Mortality of adult mice inoculated by the intracerebral route (.03 ml) with the MEF1 strain of poliomyelitis.

persistent demonstration of 2 peaks in a graph in which the time of onset of paralysis is plotted against the number of animals becoming paralyzed. This is clearly seen in Fig. 1, which was constructed from the data obtained by inoculating 152 adult mice. Although no satisfactory explanation is forthcoming, an attempt to explain the phenomenon was made by inoculating 50 mice intracerebrally with the MEF1 strain. Following this inoculation 5 mice were bled each day. The 5 specimens of sera were pooled each day and inoculated intracerebrally into 5 more mice. Virus could be demonstrated in small amounts in the serum *only* 3 days and 6 days after inoculation. These times immediately precede the 2 periods of highest incidence (Fig. 1).

Experiments with monkeys. Because of the failure to demonstrate very marked changes in the production of poliomyelitis in mice with the MEF1 strain and strains of Coxsackie viruses, similar experiments were planned using monkeys as test animals. One reason for attempting the monkey experiments was that strains of poliomyelitis virus freshly isolated from a human source could be used, whereas the MEF1 strain had been passaged many times in mice, and it was assumed that its virulence for man may have been lessened thereby. The strain of poliomyelitis selected for this work was the Cobbett strain (Stanley and Dorman) (5). Thirty monkeys were used but one monkey died from non-specific causes.

Both poliomyelitis and Coxsackie strains were inoculated in varying concentrations, 1) simultaneously and 2) at different time intervals. All inoculations were made by the intracerebral route. Of the monkeys receiving poliomyelitis virus alone 10 out of 10 became paralyzed with an average incubation period of 8.2 days. Nothing abnormal was detected with the 3 monkeys receiving Coxsackie virus alone. Twelve out of 16 monkeys receiving both Coxsackie and poliomyelitis strains became paralyzed with an average incubation period of 9.6 days. The 4 animals not showing paralysis were challenged 2 months later with 1000 ID_{50} of the Cobbett strain of poliomyelitis. All animals proved resistant to this challenge.

Discussion. It is clear that further investigations must be carried out if any definite interference between the 2 groups of viruses is to be demonstrated. Although Dalldorf(1) showed that Coxsackie viruses of group B exert a "sparing effect" on poliomyelitis infection of mice of different ages, Sulkin and Manire(2) obtained evidence that the inoculation of 3-day-old mice with the Lansing strain protected between 20% and 30% of the animals from a lethal dose of Coxsackie virus (group A). The possibility of an enhancing effect with group A strains is suggested by a close examination of Table I. No such effect could be demonstrated in monkeys. One reason may be that the concentration of group A viruses in the brain of the 2 hosts varies. Without histological evidence of infection, the ID_{50} of suckling mouse brain 3 to 4 days after infection (by the intraperitoneal route) with some group A strains may be approximately 10^{-8}. With monkeys the ID_{50} of brain tissue was 10^{-3} for the same Coxsackie strain. This was determined by removing the monkey brain 6 days after intracerebral inoculation of the virus and making an emulsion of the brain in a known volume of cold broth. This suspension was then cleared and titrated in suckling mice. Evidence that the group B strain of Coxsackie virus prolongs the incubation period of MEF1 poliomyelitis virus in adult mice is in keeping with the findings of Dalldorf(1), who demonstrated a similar phenomenon in younger mice with the Lansing strain of poliomyelitis. The failure to demonstrate interference in monkeys with group B strains confirms the observations of Howitt and Nichols(3), and of Melnick(6), but may be due to the greater resistance of monkeys to infection with Coxsackie viruses.

Summary. The intracerebral inoculation of mixtures of viruses into adult mice indicates that a group A Coxsackie virus decreases the incubation period of the MEF1 strain of poliomyelitis, while a group B Coxsackie virus increases the incubation time. Similar effects could not be demonstrated in monkeys using a strain of poliomyelitis recently isolated from the cord of a fatal human case. Attention was drawn to peculiarities in the time of onset of paralysis with mice infected with the MEF1 strain of poliomyelitis.

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