UNIVERSITY OF CINCINNATI

May 20 19 40

I hereby recommend that the thesis prepared under my supervision by Carl Edward Duffy

entitled Influence of Certain Dietary and Hormonal Factors on the Development of Resistance to Neuro-Invasiveness by Vesicular Stomatitis Virus

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

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Form 668—G. S. and Ed.—1M—7-37

INFLUENCE OF CERTAIN DIETARY AND HORMONAL FACTORS ON THE DEVELOPMENT OF RESISTANCE TO NEURO-INVASIVENESS BY VESICULAR STOMATITIS VIRUS

A dissertation submitted to the

Graduate School
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

1940

by

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A. B. Ohio University 1934

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Acknowledgment

The studies reported in this manuscript were carried out in association with Dr. Albert B. Sabin. His many valuable suggestions are deeply appreciated.

C.E.D.

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Recent investigations (1-7) with the viruses of vesicular stomatitis and equine encephalomyelitis have disclosed a type of resistance which cannot be attributed to prior exposure to the viruses nor to the presence of antiviral substances in the blood. It was shown that while the ability or inability of these viruses to invade the central nervous system (C.N.S.) subsequent to peripheral inoculation depended on the species of the host, other factors not heretofore observed were also involved. It was noted that in certain animals, the young of which are not resistant to the viruses when injected peripherally, changes occur in certain cells and tissues as the animals mature which render them refractory to the viruses when introduced by certain peripheral routes. The changes are not general in the sense that a complete resistance is established regardless of the route of injection but rather occur in definite localized areas such as in specialized nerve endings, certain regions of the C.N.S., in the blood vessels or in other tissues such as muscle. When these changes occur, since the animals remain susceptible to intracranial and intraneural injection of the viruses, it appears that the above mentioned tissue changes function as constitutional barriers which prevent the viruses from entering the C.N.S.

In view of the fact that these constitutional barriers appear as the animal matures it would be of interest to know whether, by varying certain dietary factors which are essential for a growing animal, the development of these barriers could be prevented or delayed.

Since at least one of these barriers arises coincidently with sexual maturation it was decided to investigate the influence of certain hormonal factors, which induce "precocious" sexual maturation, on the development of this barrier.

Vesicular stomatitis virus injected intramuscularly into albino mice was selected for the study for the following reasons: 1) albino mice as they grow older eventually become 100 per cent resistant to the virus when injected by the above route whereas the number of old mice which are resistant when inoculated by other routes is variable, 2) when injected by this route the virus produces readily detectable clinical manifestations of the involvement of the C.N.S. of non-resistant mice, 3) the mode of spread and pathogenesis of the virus has been worked out in considerable detail, and 4) the susceptibility of normal mice at different ages is known thus permitting correlation between the data obtained under the various experimental conditions with that already established.

The infective agent (vesicular stomatitis virus) used in the studies to be reported in this paper is classed with those obligatory intracellular parisites known as filterable viruses or simply as viruses. All viruses have the following properties: 1) they pass through filters which will retain most bacteria, 2) they cannot be seen with the ordinary microscope, 3) they multiply only in the presence of living cells.

In nature vesicular stomatitis virus causes a mild disease in horses and cattle. In these animals the lesions produced by the virus

are limited to the buccal cavity and the virus does not invade the central nervous system.

Vesicular stomatitis, caused by the virus, does not occur in nature in the albino mouse. However, when experimentally injected into young white mice the virus has a tendency to invade the central nervous system and produce a fatal ascending flaccid paralysis or a fatal encephalitis depending on the route of injection. For this reason the virus has been used in experiments dealing with immunity to virus diseases.

Since the intramuscular route of injection was used exclusively in the studies reported in this paper it might be well to consider the manner by which vesicular stomatitis virus is inhibited from entering the central nervous system of resistant mice when introduced by this route. It has been pointed out earlier that the resistance to intramuscular injection of the virus is not necessarily associated with a general resistance. Old mice which are resistant to intramuscular injection of the virus are nevertheless still susceptible to intracranial inoculation. Sabin and Olitsky (1,2) showed that although young and old mice demonstrated great differences in the degree of susceptibility to intramuscular injection of vesicular stomatitis virus they were equally susceptible to intracranial inoculation. The same authors (2) showed that the similar susceptibility of young and old mice to intracranial inoculation depended upon the manner in which the virus spread in the brain. They were able to show by microscopic study that the apparent lesions in the brain were contiguous with the ventricular system and with

the central canal of the spinal cord. Although the nerve cells of old mice were more resistant to invasion of the virus, as indicated by fewer lesions in the brains of old mice, than were those of young mice nevertheless the virus was able to gain access to the ventricles and by using a ventricular mode of spread was able to attack and multiply in a sufficient number of susceptible cells to produce a fatal encephalitis even in old mice. The above authors concluded that the equal susceptibility of young and old mice to intracranial injection of the virus was due to the ventricular or "open system" mode of spread and not to the equal susceptibility of all neural tissue. Since the lesions are limited to areas contiguous with the ventricular system and the central canal of the spinal cord it is necessary to assume that the virus is not present in the cerebrospinal fluid for if such were true one would expect to find lesions in other parts of the brain which are in contact with cerebfo-spinal fluid.

The resistance of old mice to intramuscular injection of vesicular stomatitis virus has been shown (1,3) to be dependent upon the mode of spread of the virus from the muscle to the central nervous system. The appearance of paralysis, following injection of the virus into the calf muscles of the hind leg of young mice, first in the injected leg and then in the opposite member suggests that the virus reaches the central nervous system by spreading along the nerve supply to the muscle and thus invading the spinal cord. The chief pathological lesions of the central nervous system were found in the anterior horn cells of the lumbar region of the spinal cord following the injection of the virus into the muscles

of the hind leg. These lesions consisted of various stages of necrosis and early neuronophagia. No lesions were found in the meninges or spinal and sympathetic ganglia. From the above findings it would appear that the virus, when injected into the muscle of susceptible mice, enters the peripheral nerves and by following the efferent fibers gains access to the central nervous system. Since the virus is unable to invade the central nervous system of old mice when injected into the muscles of the hind leg there must exist a barrier which holds the virus back. This barrier could be situated either in the peripheral nerves, in the specialized nerve endings or in the muscle. The barrier to neuroinvasiveness may not be in the nerve itself for as has been shown (1) the virus, when injected directly into the sciatic nerve of old, resistant mice invades the central nervous system and produces an ascending flaccid paralysis such as that seen in young mice following intramuscular inoculation. It should be noted, however, that injection of the virus directly into the nerve may place the virus in contact with broken or damaged axones and thus provide a means of entering the spinal cord.

It has been reported (1,2) that vesicular stomatitis virus is able to multiply in the muscle of 15 day old mice with the production of necrosis of the muscle cells together with hypertrophy and proliferation of the sarcolemmal nuclei. No evidence of multiplication was obtained when the virus was injected into the calf muscles of old, resistant mice and no lesions were produced.

The ability of the virus to multiply in the muscle of young mice and its inability to do so in old mice suggests that the barrier to neuroinvasiveness may be within the muscle itself. However it has been shown (4) that in the guinea pig, an animal which is also susceptible to intracranial injection of the virus, although multiplication of the virus occurred after intramuscular injection there was no evidence of invasion of the central nervous system. Here again if the muscle and nerve endings are circumvented and injection made directly into the sciatic nerve an ascending flaccid paralysis develops as in mice. Preliminary local multiplication of the virus then is not the only requisite for invasion of the central nervous system. It should be mentioned in this connection that rabies virus is able to invade a peripheral nerve without preliminary local increase (8).

A summary of the preceding discussion reveals that vesicular stomatitis virus when injected into the calf muscles of young mice undergoes local multiplication and then invades the central nervous system by way of the peripheral nerves to produce a fatal ascending flaccid paralysis. When the virus is injected into the calf muscles of old, resistant mice the virus fails to proliferate and does not invade the central nervous system. Evidently as mice grow and develop a barrier is established through which the virus cannot pass. This barrier is at present believed to be located either in the muscle, in the nerve endings or in both.

The susceptibility of white mice of various ages to intramuscular injection of vesicular stomatitis virus has been shown (1,2,7)

to be as follows: 100 per cent of 2 week old mice develop a fatal ascending flaccid paralysis. At 21 days of age 80 to 90 per cent are susceptible and at the age of 1 month 80 to 90 per cent are resistant in that they show no signs of disease. Between the 5th and 6th weeks of life about 100 per cent are refractory to the virus when injected by the above route.

Because the change from 100 per cent susceptibility to approximately 100 per cent resistance occurs between the 14th and 35th day of life and because mice suckle during the period when they are developing resistance, experiments were set up to determine: 1) the effect of the maternal diet during lactation and 2) the effect of the diet of mice weaned at 14 days of age on the development of resistant to intramuscular injection of the virus. Because sexual maturity occurs during the period when mice become approximately 100 per cent resistant to the virus an experiment to determine the effect of "precocious" sexual maturation was included in the study.

A review of the literature reveals that surprisingly little work has been done on the relationship between nutrition and host susceptibility to virus diseases. Although it has been suggested (9,10) that vitamins are important factors in immunity to virus diseases there is little experimental evidence to support such a belief. Recently Jungeblut and Feiner (11) reported that vitamin C is an effective agent for the prevention and cure of experimental poliomyelitis in monkeys. However, the findings of Jungeblut and Feiner were not substantiated by

the results obtained by Sabin (12) who found that both natural and synthetic preparations of vitamin C, administered immediately after the virus was instilled into the nose, had no effect on the course of experimentally induced poliomyelitis in the monkey. The latter author showed that monkeys whose store of vitamin C was depleted by feeding a diet deficient in the vitamin reacted in the same way as did monkeys receiving an adequate diet.

Cowdry, Lucas and Neff (13) studied the effect of a deficiency of vitamin B_1 and B_2 on the resistance of rats to herpes virus injected intracerebrally. They concluded that rats deficient in vitamin B_1 and B_2 were slightly more susceptible to the virus than were normal rats. However their data are not conclusive since the minimal lethal cerebral dose of the virus and the proper amount of fluid which should be injected were all determined during the course of experimentation. If the minimal cerbral lethal dose for the rat had been determined beforehand and kept constant during the course of the experiments the results might be more impressive.

Kuczynski (14) [quoted from Perla (15)] found that mice fed diets rich in vitamin B_1 were more resistant to the virus of yellow fever, introduced intracerebrally, than were mice fed a standard diet. Of 671 mice fed on a normal or standard diet (oats and milk) 20 per cent survived when the virus was inoculated into the brain. When mice were fed a diet rich in vitamin B_1 (wheat husks and milk) 75 per cent of 28 animals tested were resistant to intracerebral inoculation. Of 137

animals fed a diet free of vitamin B_1 for a period of 14 days only 16.7 per cent were resistant.

It appears to be justifiable to conclude from Kuczynski's data that diets rich in vitamin B_1 did enhance the resistance of mice to intracerebral inoculation of yellow fever virus. Whether his conclusion that the resistance of the nervous system to all virus infections is dependent upon the presence of an adequate amount of vitamin B_1 in the diet is justified cannot be said at the present time.

Since mice develop resistance to intramuscular injection of vesicular stomatitis virus when they are growing rapidly experiments were planned to determine whether the development of this resistance would be prevented or retarded by eliminating certain factors from the diet which are known to be essential for growth and development. In the series of experiments reported here the role of nutrition on the development of resistance was studied from two different aspects: 1) since mice suckling mothers fed a diet rich in natural foodstuffs become 80 - 90 per cent resistant at 28 days of age an experiment was planned to determine whether mice suckled by mothers fed a diet deficient in certain vitamins would also become resistant, 2) a second set of experiments was planned to determine the influence of an insufficient amount of a diet consisting of natural foodstuffs and of diets deficient in certain vitamins on the development of resistance in prematurely weaned mice.

Diets deficient in vitamin B_1 and riboflavin (vitamin B_2)

were selected for the studies primarily because these vitamins are known to be essential for growth and development (16,17). However a deficiency of either of the above vitamins gives rise to other physiological and pathological changes which may play a part in the development of resistance in the growing animal. Before taking up a discussion of the above mentioned physiological and pathological changes it might be well to discuss at this point the nature of the above vitamins. Vitamin B_1 and riboflavin (vitamin B_2) are two members of what is now termed the B complex. They are both present in yeast. Vitamin B_1 is often termed the antineuritic vitamin. It prevents beriberi in man and polyneuritis in animals. The vitamin is heat-labile being destroyed at 120°C in a neutral or alkaline medium. A common method for the preparation of a diet deficient in vitamin B_1 is to add yeast which has been autoclaved under 15 - 16 pounds pressure to a mixture of casein, lard, cornstarch and salts. However a diet made up in this manner is not only deficient in vitamin B_1 but also in other heat labile components of the B complex, such as vitamin B_4, which prevents paralysis in rats and chicks. Since the B_1 deficient diet used in the present studies was made up as indicated above it too was deficient in more than vitamin B_1. Throughout the remainder of the paper the diet which was used to study the influence of a diet deficient in vitamin B_1 will be termed "a diet deficient in the heat-labile B-complex" in order not to imply that the only deficiency was in vitamin B_1.

Riboflavin or vitamin B_2 is a water-soluble yellow-green florescent pigment. It is found in yeast and is not destroyed by

autoclaving at 120°C. It is necessary for growth in chicks and rats. It is a component of an oxidation-reduction system (Warburg's yellow enzyme)(18) in living cells. In the present studies a diet deficient in riboflavin was made up by adding rice polishings concentrate (rich in vitamin B_1) to a mixture of casein, lard, cornstarch and salts. It is evident from the composition of this diet that it is deficient in other factors of the B complex besides riboflavin. In order not to give the impression that the diet was deficient only in riboflavin this diet has been termed "a diet deficient in the B-complex except B_1."

No attempt will be made at this time to present a review of all the reported physiological and pathological changes which have been attributed to a deficiency of vitamin B_1. Only those changes which seem to be related to the present problem will be discussed. It has long been known that a deficiency of vitamin B leads to definite neuromuscular symptoms. In rats and birds these symptoms are manifested as spastic paralysis, ataxia, opisthotonus and convulsions. In addition to the above a loss of appetite occurs in animals fed a B-deficient diet with a consequent loss of weight. It is difficult in retrospect to attribute the changes described by the early workers solely to the lack of vitamin B since the rations used in the experiments were deficient in more than one essential nutrient, namely, protein (rice diets) minerals and other vitamins. The experimental animals were therefore suffering from a multiple dietary deficiency.

Later investigators have eliminated from their diets, as far as possible, only the B complex or certain of its constituents.

Stern and Findlay (19) studied the changes in the nervous system of rats fed a diet deficient in vitamin B_1. They found that degeneration of the myelin sheaths of peripheral nerves occurs when rats are fed this diet. Zimmerman and Burack (20) found that there was extensive degeneration of the myelin sheath of the sciatic nerve in dogs deprived of water-soluble B vitamins. Davison and Stone (21) also found extensive degeneration of the myelin sheath of the sciatic nerve in rats fed a diet deficient in vitamin B_1.

The fact that degeneration of the myelin sheath of peripheral nerves occurs in animals fed a diet deficient in vitamin B_1 assumes great importance from the viewpoint of the present study. It will be recalled that when vesicular stomatitis virus is injected into the muscle of resistant mice it does not invade the peripheral nerves and thus does not invade the central nervous system. If similar degeneration of the myelin sheath of the peripheral nerves occurred in mice fed a diet deficient in B_1 it might be permissable to assume that the virus could gain access to the axones of peripheral nerves and thus invade the central nervous system. On the other hand if the barrier to neuroinvasiveness is dissociated entirely from the peripheral nerves and is located within the muscle other effects of a deficiency of vitamin B_1 must be considered.

It has recently been shown that cocarboxylase is essentially a diphosphoric ester of vitamin B_1 (22). Cocarboxylase is a co-ferment which activates carboxylase. The latter enzyme is important in the carbohydrate metabolism of plant cells in that it converts pyruvic acid

to acetaldehyde and carbon dioxide. As far as the writer is aware no similar function of vitamin B_1 has been demonstrated in animal cells. However it is not beyond the realm of possibility that B_1 may function in a similar manner in animal cells. A failure of the cells of the muscle to become resistant to the virus might well accompany any disturbance in the carbohydrate metabolism.

Zimmerman, Cowgill and Fox (23) found that marked degeneration of the medullary sheaths and axis cylinders occurred when dogs were fed a diet deficient in riboflavin. However Zimmerman and Burack (20) did not observe changes in the peripheral nerves of rats deprived of the above vitamin. Evidently dogs and rats do not respond similarly to a deficiency of riboflavin. From the evidence found in the literature it is difficult to postulate how a deficiency of riboflavin might affect the peripheral nerves of mice and thus render them susceptible to intramuscular injection of vesicular stomatitis virus. However recent investigations have revealed that riboflavin is a component of an oxidation-reduction system in living cells. Riboflavin, attached to a protein molecule through a phosphoric acid nucleus, is now known to be a part of Warburg's yellow enzyme. This enzyme serves as an oxygen carrier in cell respiration. It is conceivable that a deficiency in the diet of riboflavin would consequently lead to disturbances in cell metabolism and in this manner interfere with the normal development of resistance in the growing animal.

In one set of experiments mice which had suckled mothers fed a diet deficient either in vitamin B_1 or riboflavin were tested for resistance to the virus. It is evident that while the animals were suckling they were dependent upon the mothers' milk as a source of the above vitamins. The rate at which the mother was depleted of riboflavin and vitamin B_1 would of course be determined by the vitamin intake. There appear to be no reports in the literature on the rate of depletion of vitamins in the mouse. Sure (24) has reported that lactating rats when put on diets deficient in the above vitamins on the day of parturition are depleted of their store of vitamins in about 2 weeks. In the present studies the lactating mice allowed to feed on a diet of natural foodstuffs for 2 days after delivery and were then put on the deficient diets.

The effect of a diet deficient in vitamin E on the development of resistance to intramuscular injection of vesicular stomatitis virus was studied because it has been shown (25) that when lactating rats are fed such a diet about 75 per cent of the suckling young develop paralysis between the 21st and 25th day of life. Olcott (26) and Pappenheimer (27) have studied the pathological changes in young rats due to a deficiency of vitamin E. They found that the alterations are strictly limited to voluntary muscle. The lesions in the muscles consisted of extensive degeneration of the muscle cells. Although muscular dystrophy due to a deficiency of vitamin E has not been reported in the

mouse the study was nevertheless undertaken since a lack of vitamin E seems to play some role in the well-being of muscle cells and the muscle may play a part in the development of resistance to vesicular stomatitis virus.

It has been shown (28-29) that young rats kept at constant weight by underfeeding continue to grow in that certain organs or parts increase in weight at the expense of others. The testes, suprarenals, eyeballs, central nervous system, and skeleton continued to grow while other organs either lose weight or maintain their original weight. It will be noted that musculature is not included among those organs or parts which increase in size when rats are kept at a constant weight.

It has been noted above that the inability of vesicular stomatitis virus to invade the central nervous system of old mice following intramuscular injection may be associated with the failure of the virus to multiply in the muscle. If the musculature of young mice kept on a partial inanition diet was found to react in the same manner as that of rats it might be that a retardation in the development of resistance would result if mice were underfed. An experiment designed to determine the effect of underfeeding on the development of resistance to intramuscular injection of the virus was carried out in the present series of studied. The results appear later in the paper.

It has been pointed out earlier that mice become about 100 per cent resistant to intramuscular injection of vesicular stomatitis

virus during the period when they reach sexual maturation (28-36 days of age). The writer is not familiar with any experimental studies in which an attempt was made to correlate sexual maturation and immunity to virus diseases. It should be noted however that the incidence of poliomyelitis decreases after the age of puberty. This decreased incidence might, however, be due to immunity to the disease acquired by having come in contact with subinfective doses of the virus rather than to sexual maturity..

In order to study the relationship between sexual maturation and development of resistance to intramuscular injection of vesicular stomatitis virus an experiment was carried out to determine whether mice which had been caused to become "precociously" sexually mature would be more resistant than normal mice. Pregnant mare serum was used to induce early sexual maturation. The results obtained in this experiment appear later in the paper.

Methods

Virus, animals, diets, and hormonal factor: The New Jersey strain of vesicular stomatitis virus was used in the present series of studies. In every instance freshly passaged virus was employed. The brains of mice succumbing to intracerebral injection of the virus were ground with sand and sufficient broth to make a 10 per cent suspension. The suspension was centrifuged at about 1000 R.P.M. for 5 minutes in a horizontal centrifuge. The supernatant fluid was designated as the 1:10 dilution. Two-tenths c.c. of this suspension was injected into the calf muscles of the hind leg of the mice to be tested. The minimal cerebral lethal dose (M.C.L.D.) was determined by injecting 10^{-6} and 10^{-7} dilutions of the suspension intracerebrally into groups of 3 mice (0.03 c.c. per mouse). The titer of the virus suspension was usually 10^{-7} but occasionally only 10^{-6}. Each 0.2 c.c. injected intramuscularly therefore contained from 1 to 10 million M.C.L.D.

The albino mice were of inbred stock supplied by a local dealer who raised them on the "M" diet listed below. Female mice were obtained during the 3rd week of pregnancy and delivered in the laboratory so that an accurate record of the age of the offspring was available.

Before undertaking any experiments it was necessary to determine the susceptibility of the above strain of mice at various ages. Preliminary tests showed that these mice reacted to intramuscular injection of the virus in the same manner as the mice previously studied (1,2).

"M" diet

Yellow Corn Meal	45%
Ground Wheat	20%
" Oats	12%
Alfalfa Meal	2%
Bone Meal	2%
Dried Milk (skimmed)	8%
Tankage	4%
NaCl	2%
$CaCo_3$	1%
Meat Scrap	4%
	100%

To study the influence of a deficiency of certain dietary factors on the development of resistance a purified, artificial vitamin-free diet was prepared from the following ingredients:

Casein (Vitamin-free)[1]	22%
Corn Starch	54%
Lard	20%
McCollum's Salt Mixture	4%
	100%

1. Casein Company of America

Depending upon the particular deficiency to be studied essential accessory food factors were added to or excluded from the above diet. The composition of the artificial diets used will be found in Table I.

A commercial preparation of pregnant mare serum (Gonadogen--The Upjohn Company) was used to induce "precocious" sexual maturation.

Experimental

Influence of underfeeding on the development of resistance to vesicular stomatitis virus in prematurely weaned mice: Before attempting to

Table I

Purified, Artificial Diets

Diet	Constituents					
	Vitamin-free stock diet	Dehydrated brewer's yeast[1] (autoclaved)[2]	Dehydrated brewer's yeast (untreated)	Rice polishings concentrate[3]	Wheat germ oil[4]	Cod liver oil[5]
1. Supplemented artificial	100 gms.		8 gms.		0.5 gms.	4 gms.
2. Deficient in heat-labile B-complex	100 "	8 gms.			0.5 "	4 "
3. Deficient in B-complex except B_1	100 "			6 gms.	0.5 "	4 "
4. Deficient in vitamin E	100 "		8 "			4 "

1 & 5: Mead Johnson and Company
2: 10 hours at 16 pounds
3: The Borden Company
4: Furnished through courtesy of General Mills

determine the effect of a deficiency of any single dietary factor it was decided to learn first the influence of underfeeding with a diet rich in natural food-stuffs and vitamins on the development of resistance to intramuscular injection of the virus. Since 14 day old mice are 100 per cent susceptible to inoculation by this route but become resistant during subsequent weeks the question arose whether or not mice weaned at 14 days of age and fed just enough food to maintain their weaning weight would become resistant later. To answer this question mice were taken from their mothers when 2 weeks old and fed a mixture consisting of 1500 gms. of the "M" diet, 1000 c.c. of whole milk, 150 gms. of dehydrated brewers yeast[1], and 60 gms. of Cod liver oil[2]. At the time of weaning 6 to 8 mice were placed in a metal box containing excelsior. Their individual weights were recorded daily. The amount of food given a group of mice was weighed and recorded each day so that if on an ensuing day a general gain in weight was noted the amount of food given that day was diminished. If an occasional mouse began to gain weight in excess of 7 gms. it was removed from the group and given less food for a day or two. When it had lost the excess weight it was returned to the group. In the early part of the experiment mice which were removed because of excess weight were given no food on the day of removal. This practice was discontinued because it was found that 24 hours of starvation was usually fatal to these young mice. Siblings of the underfed mice were weaned simultaneously and given the same diet ad libitum.

1. Mead Johnson and Company
2. Mead Johnson and Company

INFLUENCE OF UNDERFEEDING ON THE DEVELOPMENT OF RESISTANCE TO INTRA-MUSCULAR INJECTION OF VESICULAR STOMATITIS VIRUS

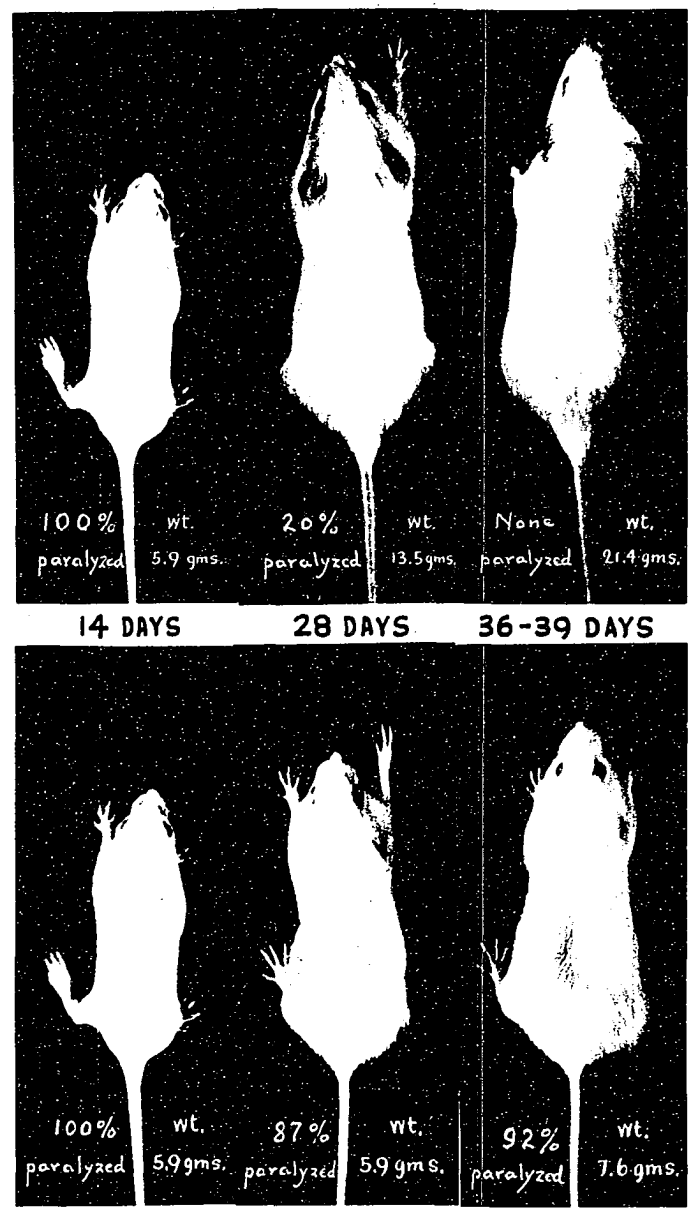


CHART I

EFFECT OF UNDERFEEDING ON DEVELOPMENT OF RESISTANCE

FIGURE 1

Growth curves of underfed and well-nourished mice weaned at 14 days

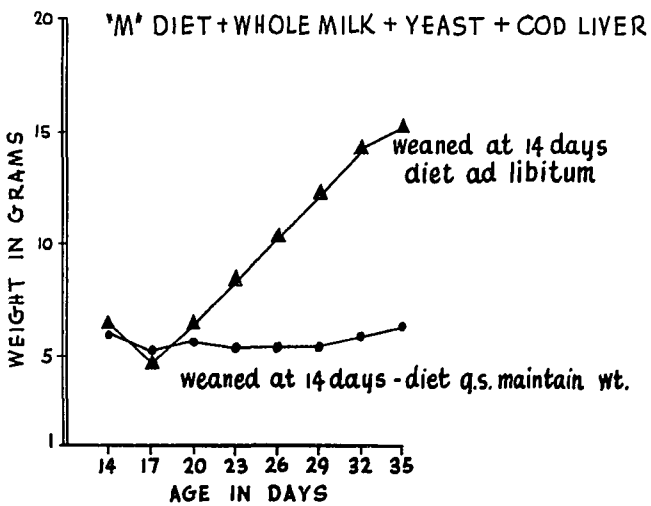
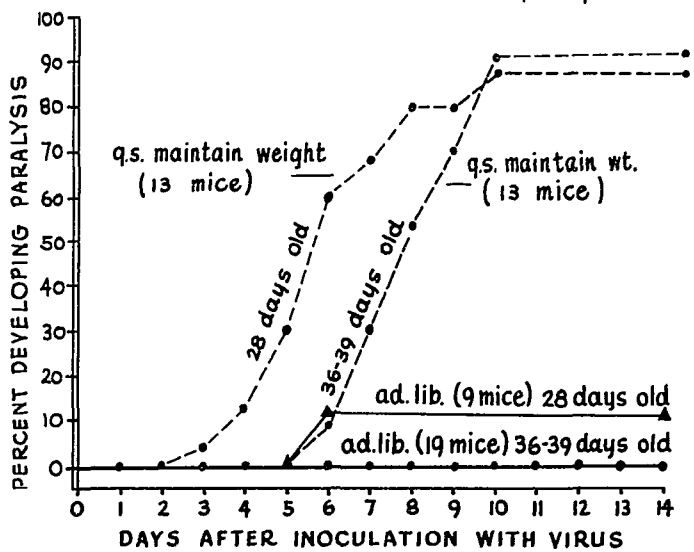


FIGURE 2

Susceptibility curves of underfed and well-nourished mice weaned at 14 days



The accompanying photographs show the appearance of the underfed and well-nourished mice at the age of 14, 28, and 39 days. It will be noted that growth has occurred in the mice held at weaning weight in that they are larger and appear more mature than they did at 14 days of age. The 28 and 39 day old mice have larger heads, their hair has grown, their eyes are larger, and the skeletal structure is more developed. When the underfed mice are compared with the well-nourished ones it is evident that there is only a slight difference in length. However, there is a considerable difference in weight. Mice weaned at 14 days of age usually lose weight for about 3 days and then begin to gain (Chart I, Figure 1). In the group which was given food ad libitum the weaning weight was attained on the 20th day of life. From that time on they gained rapidly and at 28 days of age were almost as heavy as unweaned mice of the same age.

The data obtained in these experiments are presented graphically in Chart I. The curves (Figure 2) indicate the percentage of mice which were paralyzed on different days after inoculation of the virus. The results reveal significant differences in the susceptibility of the 2 groups. At the age of 28 days, 87 per cent of the underfed mice developed paralysis as contrasted with only 11 per cent of the well-nourished animals. The difference is even more striking in the 36 to 39 day old mice. Ninety-two per cent of the underfed developed paralysis whereas 100 per cent of those given food ad libitum were resistant. When the two malnourished groups are compared it is seen that approximately the same percentage of the 28 and 36 to 39 day old mice developed paralysis. However,

the onset was earlier in the younger group. Paralysis appeared as early as the third day after inoculation in the 28 day old animals and by the 6th day 60 per cent were paralyzed. In the 36 to 39 day old group signs of C.N.S. involvement did not appear until the 6th day and by the 8th day only about 50 per cent were paralyzed.

Influence of maternal diet on development of resistance: Since mice suckling mothers on a diet rich in natural foodstuffs ("M" diet) become 80 to 90 per cent resistant to intramuscular injection of the virus at the end of the suckling period, it was decided to determine whether mice suckled by mothers fed diets deficient in the above vitamins would also develop this resistance. In this set of experiments the mothers were maintained on the "M" diet throughout pregnancy and for 2 days after delivery, when they were given the diets listed in Table I. The offspring were permitted to remain with their mothers for 28 days and then continued on the respective diets until the end of the test period. All mice were observed for at least 21 days after inoculation of the virus. Different groups of mice were tested at 4, 5, and 6 weeks of age. The data obtained in these studies are presented graphically in Chart II. Growth and

susceptibility curves of mice suckled by mothers fed diets rich in natural foodstuffs are included for comparison.

Purified, artificial vitamin-free diet supplemented by addition of yeast, cod liver oil, and wheat germ oil: A total of 41 mice, which had been suckled by 8 mothers feeding on the above diet, were tested (Chart II, Figure 3). Seventy-three per cent of 15 mice tested at 4 weeks of age proved to be resistant. The average weight of these 15 mice was 9.7 grams. At 5 weeks of age 93 per cent of 14 mice (average weight 12.9 grams) did not show evidence of C.N.S. involvement and at 6 weeks of age 100 per cent of the 12 mice tested (average weight 12 grams) were refractory. Although the mice in this group were not quite as resistant at 4 weeks of age as mice which had suckled mothers fed a diet of various natural foodstuffs (Chart II, Figure 2) they did, however, develop resistance normally in subsequent weeks.

Purified, artificial diet deficient in the heat-labile components of the B-complex: Nine mothers suckling 54 mice were put on this diet 2 days post partum. However, due to cannibalism first by the mothers and later by the more aggressive offspring only 8 of the original 54

mice were available for testing. All of these 8 mice proved to be susceptible at the age of 4 weeks (Chart II, Figure 4). The nature of the infection in these mice was different from that occurring in other mice in that only 2 developed the characteristic paralysis. That death of the other 6 was due to invasion of the C.N.S. by the virus was evident from the fact that virus was recovered from the spinal cord and brain or from the spinal cord alone of these mice at the time of death. Apparently the virus, once it had gained entrance to the C.N.S., spread rapidly to involve vital centers of the cord and medulla before paralysis could develop.

Growth was markedly retarded in these 8 mice. At 28 days of age their average weight was only 4.3 grams as compared with an average weight of 9.7 grams for 4 week old mice which had suckled mothers fed the purified, artificial diet supplemented with yeast, cod liver oil, and wheat germ oil (Chart II, Figure 1).

Because paralysis was not a constant occurrence in this group of mice, Chart II, Figure 4 indicates the day of death rather than the day of onset of paralysis.

Purified, artificial diet deficient in the vitamin B-complex except B_1: Two days post partum 9 mothers suckling 62 mice were placed on this diet. Cannibalism occurred in this group as in the previous one. However, the mortality was not so high since 33 of the original 62 mice survived

to be weaned at 28 days. At the age of 4 weeks 13 of the mice were tested, and all were susceptible to intramuscular injection of the virus (Chart II, Figure 5). At 5 weeks 90 per cent of 10 animals were resistant and at 6 weeks 67 per cent of 10 mice showed no signs of C.N.S. involvement. In this group, as in the group of mice which suckled mothers fed a diet deficient in the heat-labile components of the B-complex, growth was retarded. The average weight of the 13 mice tested at 4 weeks of age was 5.3 grams, at 5 weeks of age the average weight was 7.5 grams and at 6 weeks the average weight of 10 mice was only 8.1 grams (Chart II, Figure 1).

Purified, artificial diet deficient in vitamin E (Wheat germ oil): Two days after delivery 8 mothers suckling 56 young were placed on this diet. Forty of the young survived to be tested at 4, 5, and 6 weeks. Growth was not retarded in this group of mice (Chart II, Figure 1). At the age of 4 weeks 54 per cent of 15 mice, whose average weight was 13.6 grams, were resistant. However, at 6 weeks of age 100 per cent of 10 mice (average weight 15.4 grams) were refractory (Chart II, Figure 6).

A summary of the data in Chart II reveals that when lactating mice are fed a purified, artificial diet which has been supplemented by the addition of yeast, cod liver oil, and wheat germ oil the suckling young grow well and develop resistance to the intramuscular injection of vesicular stomatitis virus.

CHART II

INFLUENCE OF MATERNAL DIET DURING LACTATION ON DEVELOPMENT OF RESISTANCE TO INTRAMUSCULAR INJECTION OF VESICULAR STOMATITIS VIRUS IN THE OFFSPRING.

FIGURE 1

Growth curves of mice suckling mothers on various diets

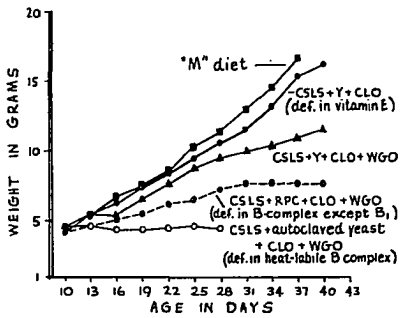


FIGURE 2

"M" diet or "M" diet + whole milk + CLO + yeast

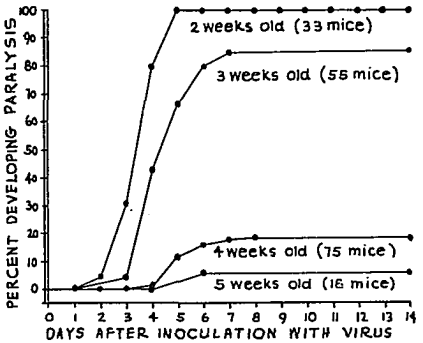


FIGURE 3

CSLS + Y + CLO + WGO

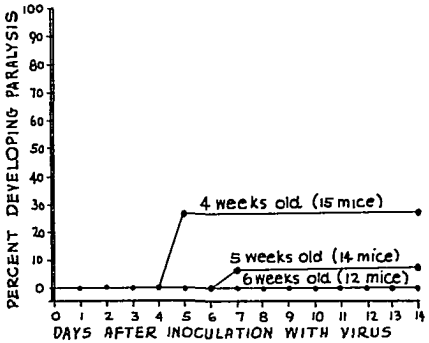


FIGURE 4

CSLS + autoclaved yeast + CLO + WGO (def. in heat-labile B-complex)

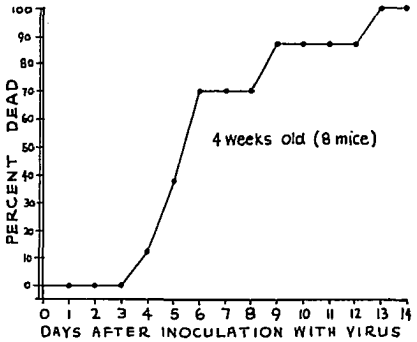


FIGURE 5

CSLS + RPC + CLO + WGO (def. in B-complex except B1)

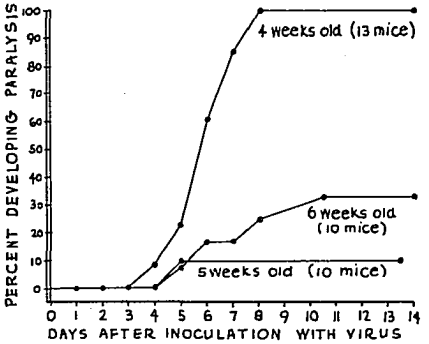
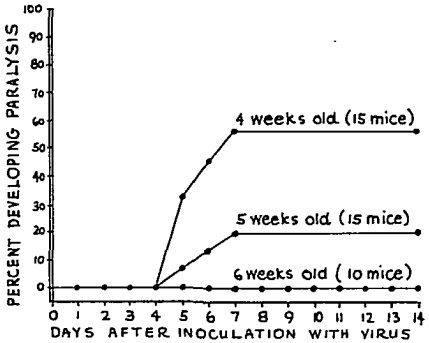


FIGURE 6

CSLS + Y + CLO (def. in Vitamin E)



CSLS = CASEIN, CORNSTARCH, LARD & SALTS (McCOLLUM'S)
RPC = RICE POLISHINGS CONCENTRATE
CLO = COD LIVER OIL
WGO = WHEAT GERM OIL
Y = DEHYDRATED BREWERS YEAST

When lactating mice are put on a purified, artificial diet deficient either in the heat-labile B-complex or in the B-complex except B_1 there is a marked retardation of growth in the suckling young and a delay in the development of resistance to intramuscular injection of the virus.

Young mice which suckled mothers put on a diet deficient in vitamin E two days after delivery, although they continued to gain weight, did not develop resistance to vesicular stomatitis virus in a normal manner.

Influence of various diets on the development of resistance in prematurely weaned mice: In this set of experiments mice which had suckled mothers maintained on the "M" diet were weaned at 14 days of age. Four groups of approximately equal numbers of siblings were given the diets listed in Table I. Different groups of mice were tested at 4, 5, and 6 weeks of age. The mice were continued on the diet until the end of the test period.

Purified, artificial diet supplemented by addition of yeast, cod liver oil, and wheat germ oil: It has been pointed out earlier that mice weaned at 14 days of age lose weight for about 3 days and then begin to gain. After the initial loss in weight the prematurely weaned mice fed the above diet grew rapidly (Chart III, Figure 1). The average weight of the 16 mice tested at 4 weeks of age was 12.3 grams. Sixty-three per cent of these 16 mice proved to be resistant (Chart III, Figure 3) to

intramuscular injection of the virus. At 5 weeks of age all of the 12 animals tested (average weight 13.2 grams) were refractory and at 6 weeks 91 per cent of 11 mice, whose average weight was 14.8 grams did not develop signs of C.N.S. involvement.

Purified, artificial diet deficient in the heat-labile components of the B-complex: A total of 39 mice were weaned at 14 days and placed on this diet. It was found that both growth and development of resistance were retarded in these animals (Chart III, Figures 1 and 4). The average weight of the 15 mice tested at 4 weeks was 8.6 grams, and only 20 per cent of these animals were found to be resistant. Fifteen mice whose average weight was 6.7 grams were tested at 5 weeks of age. Only 33 per cent were refractory. At 6 weeks of age 9 mice were tested. The average weight of this group was 10.7 grams. Sixty-seven per cent showed no evidence of C.N.S. involvement.

Purified, artificial diet deficient in the vitamin B-complex except B_1: The weaned mice which were fed this diet, like those in the previous group, failed to gain weight in a normal manner (Chart III, Figure 1). The average weight of the mice tested at 4 weeks was 8.2 grams, at 5 weeks the average weight was 8.8 grams and at 6 weeks 10.7 grams. At 4 weeks of age 67 per cent of 15 mice tested did not develop paralysis. Of the 18 mice tested at 5 weeks 67 per cent were resistant and at 6 weeks 89 per cent of 9 mice were refractory (Chart III, Figure 5). The relatively

high degree of resistance (67 per cent) of the 4 week old mice is surprising when it is recalled that 100 per cent of the mice which suckled mothers fed this diet were susceptible at 4 weeks of age (Chart II, Figure 5). It would appear that during the 14 days of suckling some factor was obtained in the milk which enabled these animals to develop resistance. It should be noted, however, that at 5 weeks of age only 67 per cent of the weaned mice fed the above diet were resistant as compared with 100 per cent of those weaned and fed the supplemented diet.

Purified, artificial diet deficient in vitamin E (wheat germ oil):

When mice were weaned at 14 days of age and put on the above diet it was found that they gained weight in the same manner as mice which were weaned and given the supplemented diet containing wheat germ oil (Chart III, Figure 1). The average weight of 15 mice tested at 4 weeks of age was 9.7 grams. Only 20 per cent of these animals proved to be resistant to the virus. At 5 weeks of age 100 per cent of 10 mice, whose average weight was 14.3 grams, were refractory. The average weight of 6 mice tested at 6 weeks was 13.8 grams. Only 33 per cent of these animals did not develop paralysis. Because only 6 six-week old mice were available for testing the apparent high degree of susceptibility of these animals cannot be considered significant until larger numbers are tested.

It appears from these data that prematurely weaned mice when fed a purified, artificial diet, which has been supplemented by the addition of vitamins, become resistant to intramuscular injection of vesicular

CHART III

INFLUENCE OF DIET OF PREMATURELY WEANED MICE (WEANED AT 14 DAYS OF AGE) ON THE DEVELOPMENT OF RESISTANCE TO INTRAMUSCULAR INJECTION OF VESICULAR STOMATITIS VIRUS

FIGURE 1

Growth curves of prematurely weaned mice fed various diets

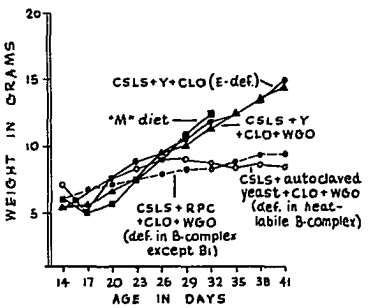


FIGURE 2

"M diet + whole milk + CLO + Y

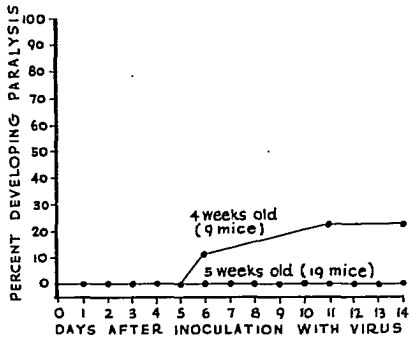


FIGURE 3

CSLS + Y + CLO + WGO

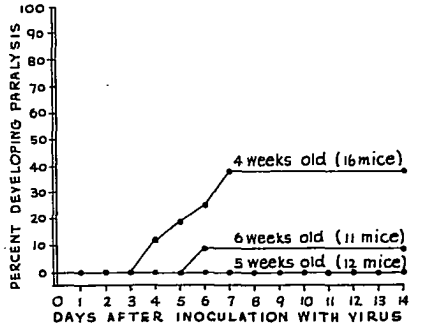


FIGURE 4

CSLS + autoclaved yeast + CLO + WGO (def. in heat labile B-complex)

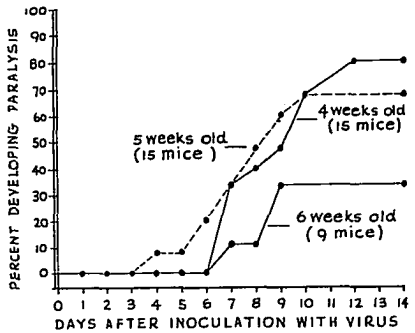


FIGURE 5

CSLS + RPC + CLO + WGO (def. in B-complex except B1)

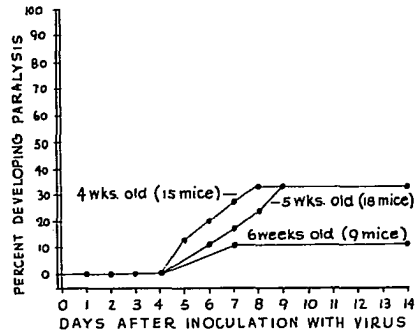
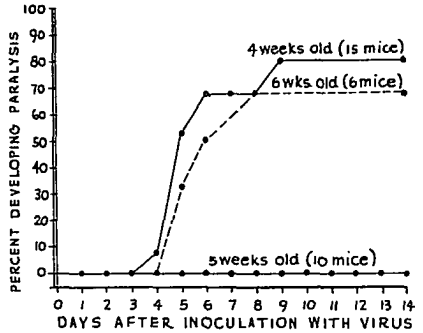


FIGURE 6

CSLS + Y + CLO (def. in vitamin E)



CSLS = CASEIN, CORNSTARCH, LARD & SALTS (Mc COLLUM'S)
RPC = RICE POLISHINGS CONCENTRATE
CLO = COD LIVER OIL
WGO = WHEAT GERM OIL
Y = DEHYDRATED BREWER'S YEAST

stomatitis virus in the same manner as do prematurely weaned mice fed a mixture of natural food-stuffs and vitamins.

When mice are weaned at 14 days of age and put on a purified artificial diet deficient in the heat-labile components of the B-complex both growth and development of resistance are retarded. Although retardation in the development of resistance was not marked in the mice weaned and fed a purified, artificial diet deficient in the B-complex except B_1 still only 67 per cent were resistant at 5 weeks as compared with 100 per cent of the weaned mice which received the supplemented diet.

In the group of weaned mice which were fed a purified, artificial diet deficient in vitamin E there was a delay in the development of resistance although growth was not retarded.

Influence of various deficient diets on the resistance of full-grown mice: The effect of dietary deficiencies on the resistance of full-grown mice was not investigated extensively in these studies. However, data was obtained from several groups of mice which had been fed deficient diets, as indicated in Table II, for a period of 6 to 8 weeks.

The data (Table II) reveal that none of the animals fed the deficient diets were susceptible to vesicular stomatitis virus following intramuscular injection. Several of the mice fed the autoclaved diet developed signs of B_1 deficiency either before or after injection of the virus and several died during the test period. However, the virus was not recovered from either the brain or the spinal cord of these mice, and it must be concluded that death was due to the vitamin deficiency and

Table II

Effect of Deficient Diets

on Resistance of Full-Grown Mice

Diet	Time on Diet	No. Mice	% Resistant
Deficient in B-complex except B_1	6-8 wks.	8	100
Deficient in vitamin E	"	7	"
*("M" diet + whole milk + yeast + cod liver oil) Ingredients mixed and autoclaved 6 hrs. at 16 lbs.	"	10	"

* Deficient in heat-labile B-complex

not to the virus. It should be pointed out that the autoclaved diet in addition to being deficient in the heat-labile B-complex was also deficient in vitamin A. Since cod liver oil was added to the diet before autoclaving the diet was probably also deficient in vitamin E.

It would appear then that once mice have developed resistance to intramuscular injection of the virus they do not again become susceptible when fed the deficient diets used in this study.

Influence of "precocious" sexual maturation on the development of resistance: The purpose of this experiment was to determine whether suckling mice injected with a gonadotropic hormone which induces "precocious" sexual maturation would become resistant at an age when most mice are still susceptible. Beginning on the 5th day of life 20 mice were injected subcutaneously with 1 unit (Cartland-Nelson) of a commercial preparation of pregnant mare serum (Gonadogen). The injections were continued until the mice were 21 days old. On the twenty-first day of life the animals were weaned and tested for resistance to the virus. Twelve normal litter-mates served as controls. Descent of the testes or opening of the vagina were the criteria used to determine sexual maturation. The mothers of these mice were fed a mixture consisting of 1500 gms. "M" diet + 1000 c.c. whole milk + 150 grams yeast + 60 gms. cod liver oil. The data obtained in this study are found in Table III.

It will be seen that opening of the vagina or descent of the testes occurred by the 21st day in 100 per cent of the treated mice while none of the untreated littermate controls showed evidence of sexual maturity.

Table III

Influence of "Precocious" Sexual Maturation on the Development of Resistance in Suckling Mice

Treatment	No. of Mice	Av. wt. at time of test	% showing opening of vagina or descent of testes at 21 days of age	% resistant
1 unit of Gonadogen daily (16 units per mouse)	20	9.8	100	25
None	12 (litter-mates of above animals)	8.9	0	25

Descent of the testes or opening of the vagina occurred as early as the 17th day of life in some of the treated animals. Mice normally become sexually mature at 28 to 36 days of age.

Although "precocious" sexual maturation was induced in 100 per cent of the treated mice these animals were not more resistant than the littermate controls. Seventy-five per cent of 21 day old mice in each group were found to be susceptible to the virus following injection into the muscle.

It appears from the data obtained in this experiment that mice treated with pregnant mare serum react to the virus in the same manner as do normal mice of the same age, i.e. premature development of resistance is not associated with "precocious" sexual maturation.

Discussion

Before beginning a discussion of the data obtained in the present experiments it will be well to restate the purpose of the studies. Vesicular stomatitis virus when injected into the calf muscles of 14 day old albino mice invades the central nervous system by way of the peripheral nerves and gives rise to a fatal ascending flaccid paralysis in 100 per cent of the animals (1,2). However as mice grow and develop they become increasingly resistant to the virus when injected by this route. At 1 month of age only 10 to 20 per cent are susceptible and between the 5th and 6th week of life they are about 100 per cent resistant (1,2,7). The present investigations were undertaken to determine whether the development

of this resistance could be influenced by certain dietary deficiencies or by inducing early sexual maturation.

It was observed that mice which were weaned at 14 days of age and kept at their weaning weight by underfeeding with a diet rich in natural foodstuffs and vitamins were still highly susceptible to the virus at 28 and 36 to 39 days of age. Siblings on the other hand, weaned at the same time and given the same diet ad libitum developed resistance in a normal manner, i.e., at 36 to 39 days of age they were 100 per cent resistant. It is thus evident that when normal growth and development are suppressed by inanition resistance to intramuscular injection of the virus does not develop in a normal manner. How is this failure to become resistant to be explained? It may be recalled that physiological changes which take place in the muscle cells of mice as they grow and develop may be responsible for preventing the virus from entering the peripheral nerves and thus gain access to the central nervous system. It was noted that the calf muscles of the underfed mice used in this particular study were much smaller than those of well-nourished mice of the same age. It might be that the suppression of metabolic activity of the muscle cells, due to a lack of nutrient material, may have interfered with the development of resistance in the muscle. However at the present time it cannot be stated definitely that the failure of the calf muscles to develop was the sole contributing cause of the failure of the underfed mice to become resistant. It has been pointed out earlier that the barrier to neuroinvasiveness following intramuscular injection

of the virus may be situated in the peripheral nerve or nerve endings. This being the case then possible changes in the peripheral nerves or in the nerve endings, due to underfeeding, must also be considered. At present it is not known whether such changes do occur in mice which are held at a more or less constant weight. However it has been reported (21) that disintegration of the myelin sheaths of peripheral nerves occurs in rats on a total inanition regimen. It should be noted that because of the nature of the diet fed these malnourished mice (high in vitamins A, B-complex, D) the animals may have received sufficient vitamins even in the small amount of food given them.

Although it has been shown that underfeeding definitely retards the development of resistance in mice to intramuscular injection of vesicular stomatitis virus the present state of our knowledge does not permit one to state by what mechanism this failure to develop resistance is brought about.

When mice were permitted to suckle mothers which had been put on a diet deficient in the heat-labile components of the vitamin B-complex 2 days after delivery they did not develop resistance to intramuscular injection of the virus. At 4 weeks of age all of the 8 mice tested proved to be susceptible. Only 2 of these mice developed the characteristic ascending flaccid paralysis but the virus was recovered from the brain and cord or from the cord alone of the other 6 at the time of death. It would appear that once the virus gained entrance to the central nervous system it spread rapidly to involve higher centers

of the spinal cord and medulla before paralysis could develop.

It will be noted (Chart II, Figure 1) that growth was markedly retarded in the mice which suckled mothers fed the diet deficient in the heat-labile B-complex. The animals did not grow after the 10th day of life (weights were taken from the 10th to 28th day). Apparently the lactating mouse is depleted of the heat-labile B-complex more rapidly than the rat since depletion apparently occurred after the mice had been on the diet for 8 days.

The apparent rapid spread of the virus in the central nervous system of the mice which had been suckled by mothers fed a diet deficient in the heat-labile B-complex suggests that the failure of these animals to develop resistance may have been associated with changes in the nervous system. As already indicated earlier in the paper vitamin B_1 seems to be necessary for the maintenance of healthy neural tissue. It may well be then that vitamin B_1 is also necessary for the normal development of the nervous system. If this is the case then it may be logical to assume that the failure of these mice to develop resistance was associated with a failure of the peripheral nerves to develop in a normal manner. It must be remembered however that vitamin B_1 may play some part in the carbohydrate metabolism of the cell and as pointed out earlier a disturbance of metabolism might interfere with the development of resistance.

A marked retardation of growth and a delay in the development of resistance to intramuscular injection of vesicular stomatitis virus

occurred in mice which suckled mothers fed a diet deficient in the B-complex except B_1. At 28 days of age 100 per cent of the mice tested were susceptible. All of these mice developed the characteristic ascending flaccid paralysis. It is interesting to note that 5 week old mice which had suckled mothers fed the above deficient diet were 90 per cent resistant. This might suggest that mice feeding on a diet deficient in the B-complex except B_1 are able to develop resistance to the virus. In view of the fact that the 5 week old mice proved to be highly resistant it is difficult to formulate an explanation for the high degree of susceptibility of the 28 day old mice. Retardation of growth may have been a factor but the difference in the average weight of the two groups (2.2 grams) would not indicate this to be the sole cause. The average weight of the 35 day old mice was only 7.5 grams. In other experiments normal 14 day old mice weighing 7.5 grams or more have been found to be susceptible to the virus when injected intramuscularly. The only apparent explanation for the failure of the 28 day old mice to become resistant is that a lack of the B-complex except B_1, caused a disturbance in tissue metabolism which in turn retarded the normal development of resistance in the muscle cells.

The growth curves (Chart II, Figure 1) of the mice suckled by mothers fed a diet deficient in the heat-labile B-complex show that growth was retarded from the 10th day of life. It would appear that in the lactating mouse the heat-labile B-complex and the B-complex except

B_1 are depleted at about the same rate.

It has been shown (16, 25) that both vitamin B_1, and riboflavin, are necessary for successful lactation in the rat. The results of this phase of the study suggest that the same is true for the mouse. Of an original group of 54 mice suckled by mothers fed a diet deficient in the heat-labile components of the B-complex only 8 were alive at the end of the suckling period, and only 33 of an original 62 mice suckled by mothers fed a diet deficient in the B-complex except B_1 survived to be weaned. It is interesting to note in this connection that complete failure of lactation occurred (33) when albino rats, fed a diet deficient in yeast, were fed supplements of vitamin B_1 and riboflavin. However, if, in addition to the above vitamins, the filtrate factor (34) (the filtrate factor prevents nutritional dermatosis in chicks) was added to the diet, successful lactation occurred.

In the present series of studies nutritional muscular dystrophy was not observed in any of the mice which were suckled by mothers fed a diet deficient in vitamin E. Evidently suckling mice and suckling rats behave differently to a deficiency of vitamin E since rats suckling mothers on a diet deficient in E develop paralysis. Although the mice suckled by mothers fed the above diet did not show any clinical symptoms of a deficiency of vitamin E, nevertheless they were highly susceptible (54 per cent) to the virus at 28 days of age. However, at 5 and 6 weeks of

age the mice had developed normal resistance to the virus. Growth was not retarded in these mice and they were normal in appearance. It would appear that factors other than growth are involved in the development of resistance. At present no explanation can be offered for the retardation in the development of resistance noted in mice which had suckled mothers fed a diet deficient in vitamin E. It might be that degenerative changes may have occurred in the muscle cells which were not manifested clinically but there is no evidence at hand to support this suggestion.

A striking difference in the degree of resistance was noted between the mice weaned at 14 days of age and fed a diet deficient in the heat-labile B complex and the animals weaned at the same age and fed a diet deficient in the B complex except B_1. The latter group developed resistance in an almost normal manner while in the former group there was a marked retardation in the development of resistance. It must be remembered that both groups of mice were suckled for 14 days by mothers feeding on a diet rich in natural foodstuffs. It would appear that when mice are allowed to suckle for 14 days and are then weaned, resistance develops even when the B-complex except B_1 is eliminated from the diet. However, when heat-labile components of the B-complex are eliminated from the diet of 14 day old mice, the development of resistance is markedly retarded. It is apparent, then, that the heat-labile components of the B-complex must be included in the maternal diet and in the diet of prematurely weaned mice if resistance is to develop normally.

The high degree of resistance at 28 days of age of the prematurely weaned mice which had been fed the diet deficient in the B-complex except B_1 is surprising when it is recalled that 100 per cent of the mice, which had suckled mothers fed the same diet, were susceptible at 1 month of age. It would appear that when very young mice (2 days old) are deprived of the B-complex except B_1, they fail to become resistant to the virus at a time when normal mice are highly resistant. However, if mice, permitted to suckle mothers feeding on a diet of natural foodstuffs, are weaned at 14 days of age and given a diet deficient in the B-complex, they become resistant in an almost normal manner.

The mice which were weaned at 14 days of age and given a diet deficient in vitamin E, although they showed no symptoms of E deficiency, were more susceptible at 28 days of age than were mice which had suckled mothers fed a diet deficient in the same vitamin. It might be concluded that the unweaned mice received some factor in the milk (perhaps vitamin E) which enabled them to become more resistant than the prematurely weaned mice. Since the mice weaned at 14 days of age and fed the diet deficient in vitamin E did not show any symptoms of E deficiency, no explanation can be offered for the delay in the development of resistance.

Full-grown mice, which are resistant to intramuscular injection of vesicular stomatitis virus, did not lose this resistance when fed a diet deficient in certain components of the B-complex or in vitamin E for a period of from 6 to 8 weeks. These findings suggest that once mice become resistant to the virus, the resistance is not affected by a deficiency of the above vitamins. It may well be that cellular changes which occur during growth and development are not reversible. The fact that several of the mice which were fed a diet deficient in the heat-labile components of the B-complex developed signs of B_1 deficiency (weakness of extremities and tremors) before or shortly after injection of the virus might suggest that even when the peripheral nerves are damaged, the virus nevertheless does not invade the central nervous system.

In order that misleading generalization may not be made from the above observations, it should be pointed out that while certain dietary deficiencies appear to influence the development of resistance to vesicular stomatitis virus when injected by the intramuscular route, it cannot be said that the resistance to all viruses would be affected in the same manner. It may also be that the resistance to vesicular stomatitis virus when injected by other routes might be affected differently.

Starkey and Leathem (35) have recently shown that pregnant mare serum is capable of producing a stimulating effect on the gonads

of 12 day old mice. They found an ovarian weight increase in the immature female mice. Follicular maturation was induced but the formation of lutein tissue predominated. They also found an increase in interstitial tissue and a resulting increase in accessory sexual organs in the immature males.

In the present study no histological studies were made of the sex organs of mice treated with pregnant mare serum. However, descent of the testes or opening of the vagina occurred as early as the 17th day when 1 unit (Cartland-Nelson) of pregnant mare serum was injected daily beginning with the 5th day of life. By the 21st day of life descent of the testes or opening of the vagina was observed in 100 per cent of the injected mice. Although evidence of "precocious" sexual maturation was obtained in all the treated mice, they were just as susceptible to intramuscular injection of the virus as were their untreated littermate controls.

SUMMARY

The present series of investigations was undertaken to study the influence of certain dietary and hormonal factors on the development of resistance, in the albino mouse, to intramuscular injection of vesicular stomatitis virus. Experiments were conducted to determine the effect on the development of this resistance of (1) the maternal diet during lactation, (2) the diet of mice weaned at 14 days of age, and (3) a gonadatropic hormone which induces "precocious" sexual maturation.

The data obtained in the dietary studies are summarized in Tables IV and V.

The results (Table IV) reveal that when lactating mice were fed a purified artificial diet which had been supplemented by the addition of yeast, cod liver oil and wheat germ oil, the offspring grew and developed resistance to intramuscular injection of the virus in the same manner as did mice which were suckled by mothers fed a diet rich in natural foodstuffs.

When lactating mice were fed a purified artificial diet deficient either in the heat-labile components of the B-complex or the B-complex except vitamin B_1, there was a retardation of growth and a marked delay in the development of resistance in the offspring.

Table IV

Influence of Maternal Diet during Nursing Period on Development of Resistance in Offspring (V. S. Virus Intramuscularly)

Diet	Age of Mice								
	4 weeks			5 weeks			6 weeks		
	% resistant	No. tested	Av. wt.	% resistant	No. tested	Av. wt.	% resistant	No. tested	Av. wt.
			Gm.			Gm.			Gm.
1. "M" diet or "M" diet + whole milk + yeast + CLO	82	75	13.4	94	18	15.0			
2. CSLS + CLO + WGO + yeast	73	15	9.7	93	14	12.9	100	12	12.0
3. " + " + yeast (deficient in E)	46	15	9.5	80	15	13.6	100	10	15.4
4. " + " + WGO + RPC (deficient in B complex except B_1)	0	13	5.3	90	10	7.5	64	11	8.1
5. CSLS + CLO + WGO + autoclaved yeast (deficient in heat-labile B)	0	8	4.3	No survivors on diet					

CSLS = casein (vitamin-free) + starch + lard + salt mixture (McCollum's)
CLO = cod liver oil; WGO = wheat germ oil; RPC = rice polishings concentrate
Av. wt. = average weight

Table V

Development of Resistance in Prematurely Weaned Mice Receiving Quantitatively or Qualitatively Inadequate Diets (V. S. Virus Intramuscularly)

Diet	Age of Mice								
	4 weeks			5 weeks			6 weeks		
	% resistant	No. tested	Av. wt.	% resistant	No. tested	Av. wt.	% resistant	No. tested	Av. wt.
			Gm.			Gm.			Gm.
1. "M" diet + whole milk + yeast + CLO ad lib.	78	9	12.4	100	19	16.8	...	...	...
2. "M" diet + " " + " + CLO q.s. maintain wt.	13	23	6.6	8	13	6.9	...	...	...
3. CSLS + CLO + WGO + yeast	63	16	12.3	100	12	13.2	91	11	14.8
4. " + " + " + RPC (deficient in B complex except B_1)	67	15	8.2	67	18	8.8	89	9	10.7
5. CSLS + CLO + yeast (deficient in E)	20	15	9.7	100	10	14.3	33	6	13.8
6. " + " + WGO + autoclaved yeast (deficient in heat-labile B)	20	15	8.6	23	15	6.7	67	9	10.7

Mice suckling mothers on "M" diet, weaned at 14 days of age
"M" diet = mixture of natural foods described in text; other abbreviations-- same as in Table IV

Young mice which suckled mothers fed a purified artificial diet deficient in vitamin E (wheat germ oil), although they continued to gain in weight, did not develop resistance to the virus in the same manner as mice which had suckled mothers fed a supplemented diet containing wheat germ oil.

Mice which had suckled mothers fed a diet rich in natural foodstuffs were weaned at 14 days of age and fed the diets listed in Table V. The data obtained in this phase of the study (Table V) reveal that prematurely weaned mice fed a diet rich in natural foodstuffs and vitamins gained in weight and developed resistance to the virus in a normal manner. However, mice weaned at 14 days given the same diet, but only in amounts sufficient to maintain their weaning weight, did not become resistant. At 36 to 39 days of age these underfed mice were still 92 per cent susceptible as contrasted with 100 per cent resistance in their siblings which received the same diet ad libitum.

Prematurely weaned mice given the purified artificial diet supplemented by the addition of yeast, cod liver oil and wheat germ oil were not quite as resistant at 4 weeks of age as the weaned mice fed the diet rich in natural foodstuffs and vitamins. However, at 5 and 6 weeks of age they had developed normal resistance.

When mice were weaned at 14 days and put on a diet deficient in the heat-labile components of the B-complex both growth and development of resistance were markedly retarded.

Although retardation in the development of resistance was not marked in the weaned mice fed a diet deficient in the B-complex except

B_1 still only 67 per cent were resistant at 5 weeks of age as compared with 100 per cent of the weaned mice which received the supplemented diet.

In the group of weaned mice which were fed a purified artificial diet deficient in vitamin E there was a delay in the development of resistance although growth was not retarded.

Full-grown mice, which are resistant to intramuscular injection of vesicular stomatitis virus, did not lose this resistance when maintained on a diet deficient either in certain components of the B-complex or in vitamin E for a period of 6 to 8 weeks.

It is apparent that a deficiency of certain dietary factors, either in the maternal diet during the nursing period or in the diet of prematurely weaned mice, can retard or inhibit the development of at least one type of constitutional barrier to involvement of the central nervous system by a neurotropic virus. However, it has not been possible to break down this resistance by the same means once it has been acquired by full-grown mice.

Evidence of "precocious" sexual maturation (descent of testes or opening of vagina) was observed on the 21st day of life in 100 per cent of mice injected with pregnant mare serum (mice usually become sexually mature between the 28th and 36th day of life). However, premature development of resistance to intramuscular injection of vesicular stomatitis virus was not associated with "precocious" sexual maturation since 21 day old treated and untreated littermates were equally susceptible.

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