creatine); this reaction is slower than the interaction of arginine and glycine. These two findings indicate that the sarcosine is demethylated first. This also is in accord with the findings of Bloch and Schoenheimer with N_{15}.

Our findings account for the other observations of Bloch and Schoenheimer that when ammonia containing N_{15} is fed the isotope is found later in the amidine $\left(-\overset{\diagup NH_2}{\underset{\diagdown NH}{C}}\right)$ nitrogen of creatine. When glycine containing N_{15} is administered the isotope is found in creatine in the sarcosine nitrogen.

The formation of glycocyamine from arginine and glycine is a new biochemical reaction which may be called "transamidination." The discovery of this reaction in the kidney (the possibility of its occurring in other tissues is now being investigated) provides direct proof that arginine and glycine are precursors of creatine.

We have found previously that glycocyamine is not methylated in the kidney; this occurs in the liver.[2] Glycocyamine is formed in the kidney. Both kidney and liver therefore participate in the formation of creatine.

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NUTRITION AS A FACTOR IN THE DEVELOPMENT OF CONSTITUTIONAL BARRIERS TO INVOLVEMENT OF THE NERVOUS SYSTEM BY CERTAIN VIRUSES

RECENT investigations[1,2] have demonstrated that as animals grow older they may develop resistance to involvement of the nervous system by certain viruses not because of immunity acquired as a result of exposure to infection, nor because of a maturation affecting the whole animal or its entire nervous system, but rather as a result of changes in certain tissues or structures which those viruses must pass before they can give rise to paralysis or encephalitis. The purpose of the present communication is to report some preliminary experiments which indicate that the nutrition of the growing animal or even that of the mother during the nursing period can exert an influence on the development of those tissue changes which serve as barriers to the invasion of the nervous system by certain viruses.

The effect of intramuscular injection of vesicular stomatitis virus in mice was selected as the indicator of at least one type of such constitutional resistance because (1) under standard conditions mice of different ages react with great regularity, and (2) the pathogenesis of the disease and spread of the virus in both young and old has already been investigated in considerable detail. Preliminary tests on the albino mice used in the present experiments were in agreement with previous observations on another stock of albino mice in that it was found that at 2 weeks of age almost 100 per cent. develop a fatal ascending paralysis, at 3 weeks 80 to 90 per cent., and at 4 weeks only 10 to 20 per cent.; at 5 weeks of age the incidence of paralysis is 5 per cent. or less, and beyond the 6th week, resistance is close to 100 per cent. Previous studies have indicated that some change in the muscle cells or nerve endings or both of the maturing animals is responsible for the resistance, since the older mice remain susceptible to intracerebral or intraneural injection of the virus.

Because the change from 100 per cent. susceptibility to approximately 100 per cent. resistance occurs between the 14th and 35th days of life, and because mice continue to suckle for about 28 days, although during the last 7 or 8 days they also eat the maternal diet, two different types of feeding experiments were designed to test the role of nutrition. In one set of experiments the mothers were maintained on standard "adequate" diets throughout pregnancy and for 2 days after delivery, when they were given the various diets indicated in Table I; the offspring remained with their mothers for at least 28 days and then continued on the respective diets until the termination of the tests. In the second set of experiments, mice, suckling mothers which were receiving standard adequate diets, were weaned at 14 days; different groups made up of approximately equal numbers of siblings were given the various diets indicated in Table II. Different groups of mice were tested for resistance at 4, 5 and 6 weeks of age by an inoculation into the calf muscles of 0.2 cc of a 10 per cent. suspension of the brains of mice succumbing after intracerebral injection of the N. J. strain of vesicular stomatitis virus; this dose contains 1 to 10 million minimal cerebral lethal doses of virus. The potency of the virus was checked in each test by intracerebral titration.

The results presented in Tables I and II can be regarded only as indicating a certain trend, since the actual percentages of resistant animals in the various groups will probably change when the work is extended on larger numbers of mice. The following indications, however, are apparent:

I. When the maternal diet during the nursing period consisted of:

(a) *An artificial, purified stock diet adequately supplemented by the various vitamins*—the offspring appeared to develop their resistance normally, *i.e.*, at the same rate as when the diet consisted of a mixture of many natural foods.

[1] A. B. Sabin and P. K. Olitsky, *Jour. Exp. Med.*, 66: 15, 1937; *ibid.*, 66: 35, 1937; *ibid.*, 67: 201, 1938; *ibid.*, 67: 229, 1938; *Proc. Soc. Exp. Biol. and Med.*, 38: 595, 1938; *ibid.*, 38: 597, 1938.

[2] A. B. Sabin, SCIENCE, 91: 84, 1940.

TABLE I

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		
	Per cent. resistant	No. tested	Av. wt.	Per cent. resistant	No. tested	Av. wt.	Per cent. resistant	No. tested	Av. wt.
			gm			gm			gm
"M" diet or "M" diet + milk + yeast + CLO	*82*	75	13.4	*94*	18	15.0	..	..	...
CSLS + yeast + CLO + WGO	*73*	15	9.7	*93*	14	12.9	*100*	12	12.0
" + " + " (deficient in E)	*46*	15	9.5	*80*	15	13.6	*100*	10	15.4
" + RPC + " + WGO (deficient in B complex except in B_1)	*0*	13	5.3	*90*	10	7.5	*64*	11	8.1
CSLS + autoclaved yeast + CLO + WGO (deficient in heat-labile B)	*0*	8	4.3	No survivors on diet					

"M" diet = dry powder consisting of yellow corn meal (45 per cent.), ground wheat (20 per cent.), ground oats (12 per cent.), alfalfa meal, 2 per cent.), bone meal (2 per cent.), dried skimmed milk (8 per cent.), tankage (4 per cent.), sodium chloride (2 per cent.), calcium carbonate (1 per cent.), and meat scraps (4 per cent.).
CSLS = vitamin-free casein (22 per cent.) + corn starch (54 per cent.) + lard (20 per cent.) + McCollum's salt mixture (4 per cent.).
CLO = cod liver oil (4 per cent.); WGO = wheat germ oil (0.5 per cent.); RPC = rice polishings concentrate (6 per cent.). Yeast = brewers' yeast (8 per cent.). Av. wt. = average weight.

(b) *The supplemented artificial diet deficient in wheat germ oil (vitamin E)*—development of resistance was retarded in some of the offspring, but at 6 weeks all were resistant. We had an opportunity to test 25 mice whose mothers had been on an E deficient diet for a still longer period, *i.e.*, since one to two weeks before delivery, and they were all resistant at 6 weeks of age.[3]

(c) *The supplemented artificial diet deficient in all the components of B complex except B_1*—all of the offspring inoculated at 4 weeks of age developed paralysis, and while an appreciable number became resistant in subsequent weeks it will be necessary to test larger numbers of mice before one can evaluate the ultimate effect of this deficiency.

(d) *The supplemented artificial diet, differing from that used in (a) only in the autoclaving of the yeast (16 lbs. for 10 hours)*—there was the greatest interference with normal development. Largely because of cannibalism practiced by the mothers and the more aggressive offspring as they grew up, only 8 mice of a group of 54 survived for the test at 4 weeks of age. All of the survivors succumbed to inoculation of the virus, but in 6 of them the disease was different from that usually observed in that they died very shortly after invasion of the spinal cord by the virus (proved by subinoculation) without showing paralysis.

II. When mice, suckling mothers which were on standard adequate diets, were weaned at 14 days of age and continued on:

(a) *A diet consisting of a mixture of many natural foods ("M" diet) supplemented with fresh whole milk (37 per cent.), yeast (6 per cent.), and cod liver oil (2 per cent.)*—there was both a good gain in weight and normal development of resistance.

(b) *The same mixture of natural foods, fresh milk and added vitamins, but only in amounts sufficient to maintain their original weight*—the mice increased in size, although not in weight, but failed to develop resistance to the spread of the virus, for even at 5 weeks of age 92 per cent. developed paralysis whereas all their litter mates which received the same diet in adequate amounts remained well.

(c) *The supplemented artificial diet*—while not quite as many mice were resistant at 4 weeks as under standard

[3] We are grateful to Dr. Karl E. Mason, of the Vanderbilt University School of Medicine, for supplying us with these mice.

TABLE II

DEVELOPMENT OF RESISTANCE IN PREMATURELY WEANED MICE RECEIVING QUANTITATIVELY OF QUALITATIVELY INADEQUATE DIETS (V. S. VIRUS INTRAMUSCULARLY)

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

Mice suckling mothers on standard diet, weaned at 14 days of age.
Abbreviations—same as in Table I.

conditions, those tested at 5 and 6 weeks of age were up to the optimum level.

(d) *The supplemented artificial diet deficient in all the components of the B complex except* B_1—there was a retardation in many mice, in that only 67 per cent. were resistant at 5 weeks as compared with 100 per cent. in the control group.

(e) *The supplemented artificial diet deficient in wheat germ oil (vitamin E)*—only 20 per cent. were resistant at 4 weeks of age. The 33 per cent. resistance observed at 6 weeks can not be regarded as significant until more mice have been tested.

(f) *The supplemented artificial diet deficient in the heat-labile components of the B complex*—the two weeks of nursing on properly fed mothers protected these mice sufficiently to prevent cannibalism and loss of life, but associated with increasing signs of vitamin B_1 deficiency, there was marked retardation in the development of resistance.

It is thus apparent that the presence or absence of certain factors either in the maternal diet during the nursing period or in the diet of actively growing young mice can promote, retard or inhibit the development of at least one type of constitutional barrier to involvement of the nervous system by a neurotropic virus. Not until the effect of adding the synthetic vitamins B_1, riboflavin or E to the respective, deficient diets has been studied will it be possible to state whether or not they, or other substances, are the factors which play a role in the development of this resistance. It should also be noted here that while inadequate nutrition could prevent or retard the appearance of this resistance in growing mice, it has not yet proved possible to break it down by the same means once it has been acquired by full-grown animals, even after they have developed signs of advanced vitamin B_1, E or riboflavin deficiencies.

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EFFECT OF ESTROGENS AND ANDROGENS ALONE AND IN COMBINATION WITH CHORIONIC GONADOTROPIN ON THE OVARY OF THE HYPOPHYSECTOMIZED RAT[1]

IN 1928 Aschheim and Zondek[2] announced their discovery of a pituitary-like gonadotropin in the urine of the pregnant woman. Administered to immature female rats or mice, it caused follicular growth and luteinization in the ovaries. Subsequent studies showed that a typical A–Z response could not be secured in the hypophysectomized animal.[3,4,5]

Of the several theories advanced in explanation of the lack of effect of chorionic gonadotropin in the hypophysectomized animal, the one most generally accepted is that the pituitary of the normal animal contributes a "complementary" factor essential for the production of large follicles and corpora lutea. The secretion of this factor is believed to be mediated through the ovary, but the nature of this principle has not been definitely identified.

In connection with experiments designed to test the effects of estrogens and androgens administered alone and in combination with gonadotropins on the gonads of the normal animal, it was of interest to extend similar studies to the hypophysectomized animal. It was thought that the use of the hypophysectomized animal would obviate any modifying influences that would necessarily be introduced by the intact animal's pituitary. What follows relates to the results secured in the hypophysectomized series treated with diethylstilboestrol, estradiol dipropionate and testosterone propionate alone and in combination with chorionic gonadotropin.

The experimental material included 44 rats, 21 to 23 days old at the time of hypophysectomy. The crystalline hormones were compressed into pellets and inserted under the skin.[6] The gonadotropin was Antuitrin "S," labeled to contain 500 R.U. in each cc. Nineteen of the treated animals were allowed a seven-day regression period prior to implantation of the pellets. In twenty animals the pellets were embedded

TABLE 1

EFFECT OF DIETHYLSTILBOESTROL, ESTRADIOL DIPROPIONATE, TESTOSTERONE PROPIONATE GIVEN ALONE AND IN COMBINATION WITH CHORIONIC GONADOTROPIN ON OVARIAN GROWTH IN HYPOPHYSECTOMIZED IMMATURE RAT*

Treatment	Number of animals	Average weight of ovaries—mg
None	5	7
Diethylstilboestrol	8	28
Estradiol dipropionate	5	13
Testosterone propionate	3	8
Antuitrin "S"	9	14
Diethylstilboestrol and Antuitrin "S"	8	103
Estradiol propionate and Antuitrin "S"	3	21
Testosterone propionate and Antuitrin "S"	3	8

* The crystalline hormones were compressed into pellets and inserted under the skin. The average daily absorption (as determined by weighing the pellets at the time of implantation and on removal at necropsy) of diethylstilboestrol varied from 130 to 170 micrograms, and 40 to 63 micrograms for estradiol dipropionate. The total dose of chorionic gonadotropin was 75 R.U., distributed over three days with necropsy 96 hours after the first injection.

[1] Supported by the Christine Breon Fund for Medical Research.

[2] S. Aschheim and B. Zondek, *Klin. Wchnschr.*, 7: 8, 1928.

[3] F. L. Reichert, *et al.*, *Proc. Soc. Exp. Biol. and Med.*, 28: 843, 1931.

[4] Y. Noguchi, *Jap. Jour. Med. Sci. and Pharm.*, 5: 104, 1931.

[5] J. B. Collip, *et al.*, *Nature*, 131: 56, 1933.

[6] The author is grateful to Dr. G. Biskind, Mount Zion Hospital, San Francisco, who generously prepared the sterol pellets.