the physiological meaning of these results. One might assume that chloroform and nembutal raised brain acetylcholine above a "normal" level, but it is equally likely that the values obtained during anesthesia more nearly approximate the "true" resting levels. Thus greater activity, whether that of the usual waking state or that of the more active, convulsive state, is paralleled by a lower brain acetylcholine content than is rest. Whether this reflects an increased rate of acetylcholine breakdown during activity cannot be said. The change observed might have been much greater if brain regions rather than whole brain had been assayed. As the experiments were done, a large change in a small region may have been masked by the diluent effect of a large volume of tissue in which acetylcholine content did not change.

It will be recalled that chloroform liberates free acetylcholine from bound precursor *in vitro*.[15] For this reason we also used the quite different type of anesthetic, nembutal. In two experiments *in vitro*, nembutal did not liberate free from bound acetylcholine in homogenized rat brain. For this reason, and because bound acetylcholine was actually increased more than the free after chloroform or nembutal, our *in vivo* results probably do not reflect simple liberation of free ester from bound precursor. It is always possible that acetylcholine may accumulate as a result of esterase inhibition. Certain depressants have been shown to have anticholinesterase activity,[2,16,17] but this is apparently not the case with chloroform or single large doses of barbiturate.[18]

Conclusions. 1. Both free and total acetylcholine content of the whole rat brain are higher after nembutal or chloroform anesthesia (diffuse diminution of activity) than in the unanesthetized animal. The free acetylcholine change is more convincing after chloroform, whereas the total acetylcholine change is more convincing after nembutal. The total acetylcholine content of the frog brain is similarly elevated after nembutal.

2. Neither free nor total acetylcholine content of rat brain differed significantly after the onset of strychnine or picrotoxin convulsions (diffuse increase of activity) from that found in quiet animals awake. Nor did strychnine alter total acetylcholine content of the frog brain.

3. With the methods used, normal whole rat brain has been found to contain about 0.7 μg of free and 2.9 μg of total acetylcholine per gram wet weight; frog brain about 4.9 μg of total acetylcholine per gram.

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Effect of Nutrition on Susceptibility of Mice to Pneumococcal Infection.

GEORGE H. HITCHINGS AND ELVIRA A. FALCO.

From the Wellcome Research Laboratories, Tuckahoe, N.Y.

Several workers have studied the effects of various diets on the susceptibility of mice and rats to induced pneumococcal infections.[1,2,3,4] For the most part, these studies have dealt with the effects of B vitamin deficiencies. The observed differences between control and experimental animals have been small, and not altogether consistent from laboratory to laboratory.

It has been found in these laboratories

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TABLE I.
Effect of Diet and Concentration of Inoculum on the Resistance of Mice to Pneumococcal Infection.

Diet	Culture conc.	No. of mice	No. surviving at day indicated 1	2	3	4	6	Avg survival days	% survival
A*	10^{-8}	24	24	20	16	15	13	4.2	54
	10^{-7}	10	10	3	3	2	1	2.0	10
	10^{-6}	56	56	18	11	10	6	1.91	11
	10^{-5}	10	10	1	0	—	—	1.1	0
B†	10^{-6}	10	10	6	4	4	4	3.2	40
C‡	10^{-6}	10	8	8	6	6	3	3.4	30
Basal	10^{-6}	52	52	50	48	46	45	5.50	86
	10^{-5}	9	9	9	8	7	7	5.2	78
	10^{-4}	14	14	13	13	13	13	5.6	93
	10^{-3}	9	8	7	5	5	5	3.8	56
	10^{-2}	10	7	3	3	3	3	2.2	30

* Purina Fox Chow.
† Rockland Mouse Diet.
‡ Stock diet of Robinson and Siegel.[2]

TABLE II.
Duration of Dietary Regimen and Change in Susceptibility of Mice to Pneumococcal Infection.

Days on basal diet*	No. of mice	No. surviving 6 days	Avg survival time, days
0	10	4	3.0
1	10	6	4.5
4	10	8	5.2
5-16	52	45	5.5

* Previous to injection. After injection all animals were maintained on the basal diet.
Dose = 10^{-6} ml culture per mouse.

that a tremendous difference in susceptibility to pneumococcal infection exists between mice which have been fed a purified diet and mice maintained on a commercial small-animal diet.

Albino mice (18 to 24 g) were injected intraperitoneally with 0.5 ml of a dilution of a 17 hour culture representing 1.0 ml of a 10^{-6} concentration. The actual number of organisms injected varied from 235 to 700 as determined by plate counts. The pneumococcus, Type I, SV-1 strain, was grown on proteose-peptone-blood broth, and was passed through mice at weekly or bi-weekly intervals.

The basal diet consisted of casein 180, salts 20, cotton-seed oil 30, cod liver oil 20 and sucrose 750 g, supplemented by thiamin chloride hydrochloride 5, riboflavin 5, pyridoxine chloride 2, calcium pantothenate 20, nicotinamide 40, p-aminobenzoic acid 20, 2,3-dimethyl-1,4-naphthoquinone 0.2 and α-tocopherol 1 mg and choline chloride 1 g per kilo of diet respectively.

Mice not on experiment were maintained on commercial diet A (Table I). Experimental mice usually were fed the diet to be tested for 5 days before the injection of the pneumococci. Experimental animals were observed over a 6 day period, and the results were recorded in terms of average survival time (maximum 6.0).

Results. Table I shows the effect of various dosages of pneumococcus culture on the production of infection in mice which had been maintained on different diets. It is seen that on diet A the mice are quite susceptible to infection, and that it is only when the culture is very dilute (10^{-8}) with an average dose of 3.5 organisms, that any considerable proportion survives the injection. A control group, maintained on this diet, was injected with the culture used for each experiment. The data of Table I (dose 10^{-6} ml) represent the summation of 7 experiments with groups of 5 to 10 mice which gave average survival times ranging from 1.4 to 3.0 days and from 0 to 20% survival at the termination of the experiment. On diet B the mice appear to be somewhat less sus-

TABLE III.
Protocol of Exp. 5, May 29, 1945.

Diet	No. of mice	No. surviving at day indicated					Avg survival, days
		1	2	3	4	6	
A*	10	10	1	1	1	0	1.4
Basal	10	10	10	8	8	7	5.0
Biotin, inositol, folic acid	10	10	10	9	9	9	5.6
10% brewer's yeast	10	10	9	9	8	5	4.6
5% 70 A.I. liver	10	10	9	7	7	6	4.5
Alcoholic extract of liver	10	10	10	9	9	9	5.6

* Purina Fox Chow.

ceptible; the average survival time, 3.2 days, is greater than any found with the same dosage for a group of 10 mice on diet A. Mice on the stock diet of Robinson and Siegel[2] are less susceptible but significantly more susceptible than those on the purified diet.

Mice maintained on the basal diet are very resistant to pneumococcal infection. Thus it was only when the concentration of pneumococcus culture reached 10^{-2}, the equivalent of about one million lethal doses for mice on diet A, that any considerable proportion succumbed to the infection. A control group of 5 to 10 mice, maintained on basal diet, was injected with the culture used for each experiment. The data of Table I (dose 10^{-6} ml) represent the summation of 8 such experiments which gave average survival times ranging from 4.4 to 6.0 days and from 60 to 100% survival.

The protocol of a representative experiment is given on Table III. All animals were injected with 0.5 ml aliquots of the same culture dilution representing 10^{-6} ml of a 17 hour blood broth culture. Animals were caged in groups of 5, with 2 groups on each diet. Injections were completed within 45 minutes. However, to distribute evenly the effect of any possible change in the culture during this period, one group of five on each of the diets was inoculated before the second group on any diet was injected. The composition of the diets is given in Table IV.

The time of pretreatment on the basal diet to effect a change in susceptibility was determined in the experiments reported in Table II. It appears that if the mice are changed from diet A to the basal diet at the time of injection some increased resistance to the infection may result, while even a single day on the basal diet definitely increases the resistance.

In Table III are shown the results of a number of experiments designed to test the effect of various dietary supplements to the basal diet. In every instance the average survival time of the group of mice fell within the range of survival times found for groups of 5 to 10 mice on the basal diet. In only one instance, that of the diet in which 5% of the 70% alcoholic insoluble fraction of liver was incorporated, was the average survival time low enough to indicate that further investigation would be desirable. It is apparent that the difference between the basal and the commercial diet cannot be attributed to any known vitamin or, in fact, to any known dietary essential.

Discussion. A number of possible explanations exist for the difference in susceptibility to pneumococcal infection of mice on the two types of diet. These theories fall into two main groups. Either a protective factor in the purified diet or a factor in the commercial diet which increases the susceptibility might be postulated. The first appears unlikely. The latter explanation would be best fitted to explain the results to date. On this hypothesis some factor essential to the rapid multiplication of the pneumococcus, but not necessarily an absolute requirement for growth, would be absent from, or in low concentration in, the synthetic diet. This hypothetical substance (or substances), would not be essential for the mouse. It would, in fact, be present in minimal concentration in the blood and body fluids of

TABLE IV.
Effect of Various Supplements to the Purified Diet on Susceptibility of Mice to Pneumococcal Infection.

Supplement to basal diet	No. of mice	No. surviving 6-days	Avg survival time, days	% survival
Biotin, inositol*	5	5	6.0	100
Biotin, inositol, folic acid†	20	18	5.7	90
Alcoholic extr. of liver‡	10	9	5.6	90
75% corn starch	11	8	5.2	73
3% salts	6	6	6.0	100
12% casein	10	8	5.3	80
12% proteose-peptone	10	9	5.7	90
2% 70 A.I. liver§	10	10	6.0	100
5% 70 A.I. liver	10	6	4.5	60
10% brewer's yeast	20	15	5.3	75
5% asparagine	10	10	6.0	100

Each supplement was incorporated into the basal diet by replacement of an equal weight of sucrose.

* 40 mg inositol, 400 μg biotin per kg diet.

† 2 mg biotin, 80 mg inositol, and 15 g folic acid concentrate (equivalent to 6 mg of folic acid $\pi = 40{,}000$) per kg diet.

‡ Fraction soluble in 70% alcohol equivalent to 0.8 g liver per g diet.

§ Fraction insoluble in 70% alcohol.

Dose = 10^{-6} ml culture per mouse.

the mouse, except insofar as higher levels were maintained by a more or less continuous supply from exogenous sources. Such an hypothesis would explain the rapid change in susceptibility of the mouse which occurs when the dietary regimen is changed.

The conclusions to be drawn from the present experiments may or may not be applicable to other types and strains of pneumococci. However, Robinson and Siegel[2] found mice on all purified diets to be less susceptible to pneumococcal infection than those on their stock diet. The data reported here show similar comparative effects from their stock diet and the purified diets. It seems likely, therefore, that the same factors influencing susceptibility may be operative in both laboratories.

Summary. The resistance of mice to the intraperitoneal injection of Type I pneumococcus was found to be markedly dependent on the type of diet on which they were maintained. Those on a purified diet survived the equivalent of 100,000 lethal doses for those on a commercial laboratory diet. This difference could not be ascribed to any known dietary essential.

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Further Purification of the Follicle-Stimulating Hormone and Its Effect in Normal and Hypophysectomized Rats.*

W. H. McSHAN AND ROLAND K. MEYER.

From the Department of Zoology, University of Wisconsin, Madison.

Follicle-stimulating hormone preparations have been obtained previously by the digestion of an aqueous extract of sheep pituitary powder with trypsin.[1,2] The extracts ob-

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