in prophylaxis, are discussed. Authors' summary.

The authors acknowledge with gratitude the kind cooperation of Dr. Huntington Williams, Commissioner of Health, and Dr. Myron G. Tull, Dr. Margaret Smith and Miss Helen Zepp, all of Sydenham Hospital, Baltimore; of Miss Kathleen Foley and members of the medical staff of the Johns Hopkins Hospital, and of Dr. J. Winfred Davis, Dr. C. Howe Eller, Dr. C. LeRoy Ewing and the members of their staffs, all of the Baltimore City Health Department, in collecting cultures and information for these studies. Valuable technical assistance was also given by Dr. Evelyn A. Mauss and Miss Elaine Updike of the Department of Bacteriology, Johns Hopkins School of Hygiene.

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Effect of Corn Grits on Nicotinic Acid Requirements of the Dog.*

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Pellagra has long been associated with the ingestion of relatively large amounts of corn. The early theories regarding this relationship included suggestions that corn contained toxic materials or acted as a carrier for pathogenic organisms. Goldberger and coworkers[1] clearly demonstrated that pellagra was caused by the deficiency of a dietary factor in certain foods commonly consumed by pellagrins, and in 1937[2] nicotinic acid was found to be the vitamin supplied in inadequate amounts. However, as analytical methods have been perfected, it has become apparent that on a dry weight basis, corn contains as much, or more, nicotinic acid as eggs, milk, polished rice, oats, and rye. Furthermore, Handler[3] has found that the blacktongue syndrome in dogs is more readily produced with diets containing corn than with purified rations which contain much less nicotinic acid. In addition, we have observed[4] that corn exerts a marked growth retardation in rats which is counteracted by nicotinic acid therapy. Thus there is some characteristic of corn aside from its low nicotinic acid content, which tends to increase the organism's requirement for this substance.

Corn grits were used in the present study since they comprise a large portion of the dietary of many people in certain sections of this country, and since corn grits as prepared and marketed, usually contain significantly less nicotinic acid than does whole corn. The effect of corn grits on the nicotinic acid requirement of the dog was determined by feeding a nicotinic acid-low synthetic ration so modified as to include a high percentage of corn grits.

Experimental. The experiments were conducted with growing dogs which had been kept since weaning on the nicotinic acid-low synthetic ration reported in previous studies.[5] All

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

This work was supported in part by a grant from the National Dairy Council, on behalf of the American Dairy Association.

We are indebted to Merck and Company, Inc., Rahway, N. J., for the generous supply of crystalline B vitamins, to Abbott Laboratories, North Chicago, Ill., for the supply of halibut liver oil, and to Dr. Elmer J. Lease, Clemson Agricultural College, Clemson, S. C., for the supply of corn grits used in these experiments.

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3 Handler, P., PROC. SOC. EXP. BIOL. AND MED., 1943, **52**, 263.

4 Krehl, W. A., Teply, L. J., and Elvehjem, C. A., *Science*, 1945, **101**, 283.

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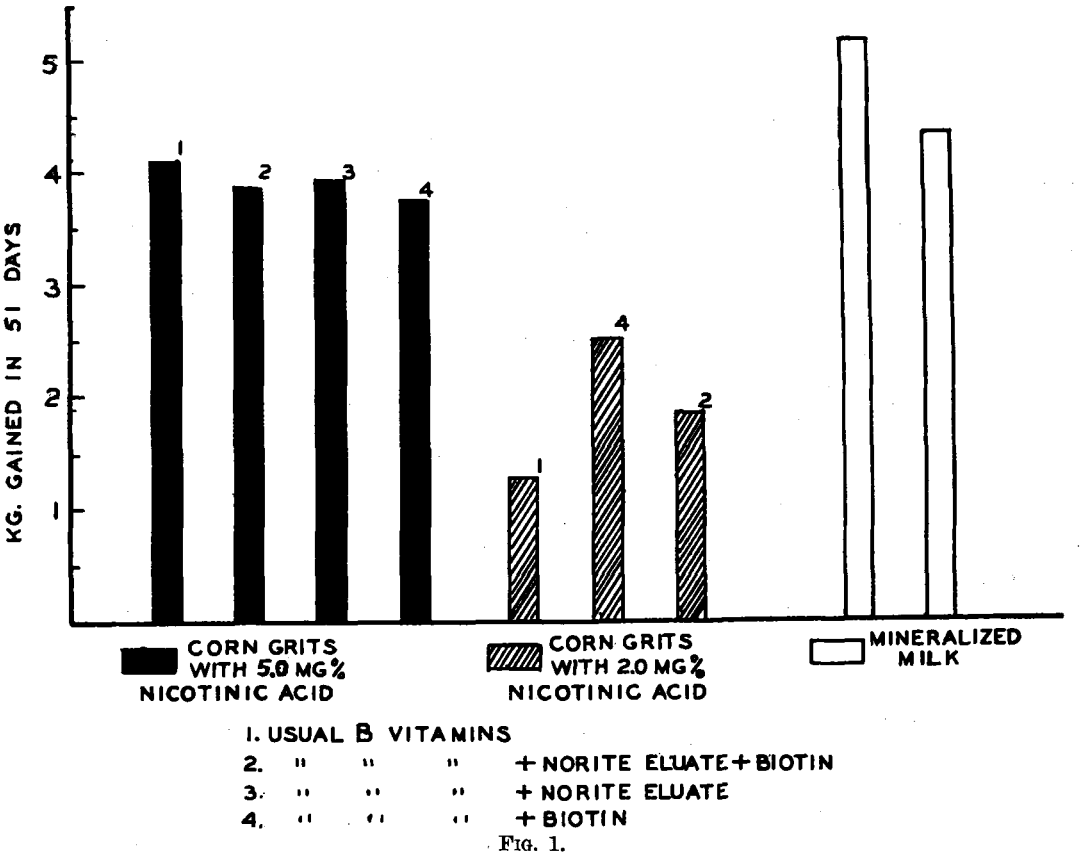

FIG. 1.
Growth of dogs on corn grits rations containing 2 levels of nicotinic acid.

of the dogs had undergone one or more attacks of blacktongue, but were in a good nutritional state with respect to nicotinic acid when placed on the experimental regime. Each dog was given 5 mg of nicotinic acid 1 week prior to starting the experiment.

The corn grits used and the synthetic basal ration were assayed microbiologically[6] for nicotinic acid. The basal ration contained 0.064 mg %. Four lots of unenriched corn grits had 0.93, 0.56, 0.62 and 0.60 mg % of nicotinic acid while one sample each of whole white corn and whole yellow corn contained 2.25 and 2.28 mg % respectively, which is a fair average for whole corn of either variety. Two lots of enriched corn grits contained 4.9 and 6.1 mg % of nicotinic acid which is considerably higher than the average whole grain value. In all cases, unless otherwise indicated, the corn grits were incorporated into the synthetic ration at a level of 60% at the expense of sucrose. This procedure insured an adequate protein level of about 24% for the entire ration. Generous amounts of the usual B vitamins except nicotinic acid (*i.e.*, thiamine chloride hydrochloride, riboflavin, pyridoxine hydrochloride, calcium pantothenate, and choline chloride) were fed by pipette. The ration was fed *ad libitum*. Vitamins A and D were given by dropper in the form of halibut liver oil.

The nicotinic acid content of the corn grits in the ration fed to one group of 4 dogs was adjusted by adding enough nicotinic acid to allow a level of 2.0 mg %, whereas the nicotinic acid content of the corn grits in the ration fed a second group of 3 dogs was increased in a like manner so as to contain 5.0 mg %. These two levels conformed to the nicotinic acid present in whole corn and enriched corn grits. Based on a daily food consumption of 35 g per kg, the two groups of

[6] Krehl, W. A., Strong, F. M., and Elvehjem, C. A., *Ind. Eng. Chem., Anal. Ed.*, 1943, **15**, 471.

dogs received 0.42 and 1.05 mg of nicotinic acid per kg of body weight per day respectively. The lowest level of nicotinic acid fed therefore was at least 25% higher than the minimum requirement for the growing dog as established by Schaefer *et al.*[7] using a synthetic ration. The two groups of dogs were kept on experiment for 51 days. The results (Fig. 1) show that the average growth for the 4 dogs on the higher level of nicotinic acid was 3.9 kg for the total period or 77 g per day, while the group average at the lower level was 1.88 kg or about 37 g per day. As a matter of comparison, 2 similar dogs on a mineralized whole milk diet grew an average of 4.75 kg in a like period of time. Whole milk contains approximately 0.7 mg % of nicotinic acid on the dry weight basis.

Since the norite eluate appears to be involved in the blacktongue syndrome in dogs[5] and since biotin has been indicated as a vitamin for the dog,[8] the effect of these vitamins, either alone or in combination was tested by supplementing these materials as indicated in Fig. 1. The growth results indicate no significant effect from either of these supplements and it appears that the nicotinic acid level is the predominating factor involved.

In an additional experiment, 2 dogs were placed at weaning on the corn grits ration in which the nicotinic acid level of the corn grits was adjusted to 1.0 mg % (the upper range for unenriched corn grits). This would furnish a daily calculated intake of about 0.21 mg of nicotinic acid per kg of body weight. Both of these dogs lost weight and died in 29 and 34 days.

In a final experiment the ration fed contained only 36% of corn grits (at the expense of sucrose) instead of the usual 60%. The corn grits in this case were enriched to such a level that the entire ration contained the equivalent of 1.6 mg % nicotinic acid as compared to 3.0 mg % for the previous 60% enriched corn grits ration. This ration was fed for a period of 30 days, during which time the dog gained 1.95 kg. This is a growth rate of about 65 g per day which is quite comparable to the 77 g per day average obtained with dogs receiving corn grits containing the higher level of nicotinic acid. Thus when the level of corn grits was reduced from 60 to 36% one half the amount of nicotinic acid gave approximately the same growth as was previously observed.

Discussion. The evidence which is accumulating, such as Aykroyd's study with humans,[9] Handler's findings with dogs,[3] our observations with rats,[4] and the present report on dogs indicates that corn significantly increases the nicotinic acid requirement. This deleterious effect might be explained in a number of ways.

On synthetic diets, there may be some beneficial synthesis of nicotinic acid which is diminished by the addition of corn grits; a substance (or substances) may be present in corn which combines with nicotinic acid to make it unavailable, or compounds may be present in corn which compete with nicotinic acid or its amide in body tissues. Koser[10] has demonstrated that a number of bacteria destroy nicotinic acid and it is possible that corn may promote and support a flora which includes such microorganisms in large numbers. Regardless of the explanation for the deleterious effect of corn we have shown that the addition of adequate amounts of nicotinic acid completely counteracts this untoward action.

While it appears that corn tends to increase the nicotinic acid requirement, foods such as milk, which contain much less nicotinic acid, tend to decrease the requirement. This is undoubtedly due to the establishment of an intestinal flora which favors the synthesis of nicotinic acid.

Two approaches to the solution of the problem of corn as it affects the nicotinic acid requirement might be to enrich corn grits and other milled corn products with nicotinic acid considerably above the whole grain level, or, as Burkholder suggests,[11] by the development of varieties of corn which are markedly higher in their nicotinic acid content.

[7] Schaefer, A. E., McKibbin, J. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 679.

[8] Smith, S. G., *Science*, 1944, **100**, 389.

[9] Aykroyd, W. R., and Swaminathan, M., *Ind. J. Med. Res.*, 1940, **27**, 667.

[10] Koser, S. A., and Baird, G. R., *J. Inf. Dis.*, 1944, **75**, 250.

[11] Burkholder, P. R., McVeigh, I., and Moyer, D., *Yale J. Biol. Med.*, 1944, **16**, 659.

Summary. When unenriched corn grits are incorporated in a synthetic ration to the extent of 60% of the sucrose, the nicotinic acid requirement of the dog is markedly increased and good growth does not result until the nicotinic acid content of the corn grits is increased to about 5.0 mg % (more than 5 times the unenriched level) which furnishes the dog with about 3 times as much nicotinic acid as is required for comparable or better growth on a synthetic or whole milk ration.

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Electrical Activity of Acetylcholine at the Phase Boundary Between Cholesterol and Sodium Chloride Solution.

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In previous papers[1,2] we have reported that acetylcholine chloride added to physiological salt solution produces a potential difference between that solution and various oils (nitrobenzene, cresol, guaiacol, etc.) so that the negative pole is on the side of the acetylcholine added.

In the apparatus an oil layer separates 2 layers of 0.9% NaCl solution. The experiments are attempts to duplicate in a simple model the potential differences found in the nervous system. Our object is to explain the potential that gives rise to the nerve impulse.

In the models previously described[1,2] the oils used were chemically different from the lipids of the plasma membrane of the living nerve. The electrical measurements with our oil cell technic cannot be done with pure cholesterol directly since this is a solid. The cholesterol must be dissolved in a water-immiscible yet conducting solvent which must not itself establish an electric negativity with acetylcholine. Such solvents are hard to find. It was found that benzyl alcohol gave no potential with acetylcholine in concentrations as high as 2%. In other words, no potential difference was measured between the two interfaces of benzyl alcohol and sodium chloride solution when acetylcholine was added to the latter on one side.

TABLE I.
Phase Boundary Potential Difference Between Acetylcholine in Saline Solution in Contact with Benzyl Alcohol Containing 0.6% Cholesterol and Pure Saline.

Conc. of acetylcholine %	Potential difference between interfaces. Oil in contact with saline containing acetylcholine is negative.
0.05	6.0 millivolts
0.10	10.0 "
0.15	14.0 "

All experiments were performed at room temperature (25°C).

The low solubility of cholesterol in benzyl alcohol (precipitation occurs at concentrations above 0.6%) may explain the small magnitude of the acetylcholine potentials in these mixtures (Table I). It is a remarkable fact that 0.05% acetylcholine can produce a negativity of 6 millivolts at a phase boundary between a saline and an oil layer which is 99% inert (benzyl alcohol).

The nervous system contains many other lipids besides cholesterol—especially lecithin. It was found that 4% lecithin dissolved in the inactive solvent benzyl alcohol produced an electric negativity of 13 millivolts when 0.5% acetylcholine was added to the saline at one side of the oil layer. Unfortunately lecithin emulsifies in the aqueous layers, which makes the potential difference unstable.

The experiments described above demon-

[1] Beutner, R., and Barnes, T. C., *Science*, 1941, **94**, 211.

[2] Beutner, R., and Barnes, T. C., *Biodynamica*, 1942, **4**, 47.