contrary to the effects of repeated doses of syncurine on grip strength as reported by Macfarlane *et al.* (*4*). This may explain in part some of the clinical difficulties experienced with syncurine, since repeated doses are less effective in producing relaxation and at the same time respiration continues to be depressed.

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Liquid Nitrogen as a Tool for Obtaining Homogeneous Bacterial Suspensions

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In studies on the physiology, serology, and pathogenicity of microorganisms, uniform suspensions are necessary but frequently difficult to obtain. In some serological work with *Erysipelothrix rhusiopathiae* we experienced difficulty because of autoagglutination. In a search for better methods of preparing satisfactory antigens for agglutination studies, the use of liquid gases suggested itself. Liquid nitrogen was chosen because of its temperature, its chemical inertness, and its rapid evaporation. Other liquid gases would probably serve as well.

The method used for preparing suspensions was as follows: A washed suspension of killed organisms was centrifuged, the supernatant liquid removed, and the organisms transferred to a mortar. A small amount of liquid nitrogen was added, and the frozen organisms were ground with a pestle until they had thawed. The procedure was repeated, and the bacteria were resuspended in saline. Occasionally the organisms were dried by washing with cold acetone before being subjected to liquid nitrogen treatment.

As compared to controls, the preparations obtained by this method were more finely dispersed, and the organisms remained in suspension for long periods.

Living cultures of mycobacteria, both virulent and avirulent, harvested from slants of Petragnani medium, were successfully suspended by the same method. Microscopic examination showed clumping to be negligible. The organisms retained their viability as shown by growth on artificial media.

By this method we have also obtained homogeneous suspensions for other serological tests, for physiological studies, and for animal inoculation. Erythrocytes treated with liquid nitrogen were completely lysed, and from such cells large quantities of stromata could be obtained by centrifugation.

The rapid evaporation, the low temperature, and the chemical inertness of liquid nitrogen make it a valuable agent for producing homogeneous suspensions of bacteria.

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Free Selection of Nutrients by Hereditarily Obese Mice

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The production of a strain of mice throwing animals that show hereditary obesity has been recently described (*1*). It may be useful to recall that they originated from the crossing of "V stock" males to offspring of "V stock" males and "C57BL/6" females. The strain (Ob ob) throwing obese animals presents a variety of characteristics corresponding to V stock genes: "nonagouti," "leaden," "piebald spotting," "waltzing," and "waved-1," as well as the "fuzzy" gene.

Very marked differences in weight between "obese" and "nonobese" members of this strain are soon apparent. For example, young adult obese mice weigh 38–56 g, whereas the weights of young adult nonobese mice are in the 16–26-g range.

In order to discover a lead to possible nutritional and metabolic abnormalities associated with this hereditary obesity, a free-selection experiment was instituted, using 10 obese and 7 nonobese animals. The animals were placed in individual screen-bottomed cages at constant temperature and humidity. They received three "diets," I, II, and III, representing essentially pure fat, carbohydrate, and protein fortified with minerals and vitamins. Diet I consisted of casein, 75%; dried defatted liver powder, 15% (representing 90% of the total calories as protein); corn oil, 5%; cod liver oil, 1%; salt 4%. Diet II consisted of sucrose, 90% (representing 90% of the total calories as carbohydrate); corn oil, 5%; cod liver oil, 1%; salt, 4%. Diet III consisted of lard, 57%; corn oil, 15%; cod liver oil, 2% (representing 90% of the total calories as fat); casein, 15.5%; dried defatted liver powder, 3.0%; salt, 7.5% (*2*). In addition, the following vitamins were added to all

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TABLE 1

GRAMS OF DIETS I, II, AND III EATEN BY OBESE AND NONOBESE MICE

Obese animals			Nonobese animals		
Diet I (high protein)	Diet II (high carbohydrate)	Diet III (high fat)	Diet I (high protein)	Diet II (high carbohydrate)	Diet III (high fat)
1.12 ± 0.555*	1.96 ± 0.644	1.73 ± 0.379	1.42 ± 0.634	2.49 ± 0.828	0.56 ± 0.396

* The figures following ± signs represent standard deviations.

TABLE 2

DAILY AVERAGE TOTAL CALORIES CONSUMED AND PERCENTAGES DERIVED FROM PROTEIN, CARBOHYDRATE, AND FAT BY OBESE AND NONOBESE MICE

Obese animals				Nonobese animals			
Total cal	Protein	Carbohydrate	Fat	Total cal	Protein	Carbohydrate	Fat
25.48 ± 5.31*	19.98 ± 4.08	27.80 ± 8.30	52.22 ± 6.59	20.44 ± 4.37	26.40 ± 9.17	44.81 ± 16.1	28.78 ± 8.35

* The figures after the ± signs represent standard deviations.

three diets in proper amounts: choline, thiamine, pyridoxine, riboflavin, niacin, calcium pantothenate, *p*-aminobenzoic acid, α-tocopherol, and inositol. These diets were placed in separate 10-cc beakers disposed on the inside of V-shaped racks, with a slit opening allowing the animals to eat but preventing them from either spilling or contaminating the diet. Food and water were given *ad lib*. Food cups were weighed daily. The animals were on experiment an average of 18 days. The results, in grams, of diets I, II, and III eaten by obese and nonobese animals daily, as well as total average daily food intakes, are given in Table 1. Total daily calories, as well as percentage derived from carbohydrate, protein, and fat, are given in Table 2.

It will immediately be apparent that very marked differences were found between obese and nonobese animals. First, evidently, obese animals ate significantly more than nonobese animals ($P = 0.03$). Although the average difference of 5 cal/day between obese and nonobese animals may not appear sufficient to account for the differences in weight observed, it may be useful to recall that in 30 days a difference of 5 cal daily, with a maximum thermochemical efficiency of 24% (*3*), could represent up to 4 g of depot fat alone. If the difference in body composition comprises proteins and associated water, the difference in body weight could in theory run up to four times this value. Second, there were equally marked differences in the proportion of the calories derived from fat, protein, and carbohydrates. The obese animals ate proportionately more fat ($P < 0.0001$), less protein ($P = 0.03$), and less carbohydrate ($P = 0.007$) than the nonobese animals, with the differences in fat and carbohydrate particularly striking. These in turn led to two important considerations. First, the increase in fat and decrease in carbohydrate intake could be easily correlated with the "glucostatic" theory of the regulation of food intake (*4*). Second, the type of diet selected by the obese animals was reminiscent of diets restricted in carbohydrate often used in the dietary management of diabetes. Both suggestions have been actively explored, and the question of diabetes is considered in another note published in this journal.

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Hereditary Diabetes in Genetically Obese Mice

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The preceding note (*1*) has described the development of a strain of mice presenting a high proportion of hereditarily obese animals and their genetic constitution. It has been shown how the obese animals, placed on a "free-choice" diet, chose a "diabeticlike" regimen, whereas the nonobese animals chose a more normal distribution of nutrients. These results led to an investigation of the carbohydrate metabolism of these animals. It may be indicated at the outset that such a study presented special difficulties on two counts. First, this being a new strain of mice, experimental animals are still produced in limited numbers; second, glucose determinations by the Somogyi-Nelson

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