The possibility existed that the 3 most stimulatory vitamins were, in reality, essential to growth of the organism, and that small amounts were being supplied as contaminants from other constituents of the medium. In order to test the possibility that the vitamins used were cross-contaminated, a complete lot of new vitamins was opened and the tests were repeated several times, with the same results as above. If contamination were involved, it seems likely that it came from some constituent of the medium besides the other vitamins. Alternatively, the organism may be capable of synthesizing pantothenic acid, folic acid, and riboflavin at sub-optimal rates which permit limited growth.

Purine and pyrimidine requirements. The omission of all purines and pyrimidines from the otherwise complete medium resulted in growth failures. It was found that individual addition of adenine, guanine, xanthine, hypoxanthine, adenosine, guanosine, or xanthosine supported heavy growth of *M. freudenreichii*. Thymine, uracil, cytosine or thymidine were incapable of supporting growth. It appears, then, that the requirement of the organism can be met by any purine or its riboside, but not by pyrimidines or their pentosides.

Carbohydrate requirements. Out of 21 carbohydrates tested, heavy growth was supported by glucose, maltose, trehalose, fructose, mannose, sucrose, lactose, starch, and galactose. It thus appears that the organism can use a wide variety of carbohydrates as its energy source.

Summary. The nutritional requirements of *Micrococcus freudenreichii* 8459 for amino acids, vitamins, purines, pyrimidines, and carbohydrates have been studied. Three complete mixtures of amino acids gave considerable variation in growth, indicating that the organism is sensitive to the ratios of the various amino acids present. Eight amino acids were indispensable for growth. No single vitamin was shown to be essential, but growth was light in the absence of pantothenic acid, riboflavin, or folic acid. The single addition of any of the purines or purine ribosides tested supported heavy growth, but none of the pyrimidines or their pentose derivatives tested permitted growth in the absence of a purine. Of 21 carbohydrates tested, 9 were capable of serving as an energy source for *M. freudenreichii*. Growth was greatly improved when CO_2 was supplied to the medium as $NaHCO_3$.

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Effects of High Fat and Carbohydrate Diets on Rat Liver Phosphatase.* (23491)

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The fact that adaptation of rats to a high fat diet increases the rate of utilization of fat and decreases that of carbohydrate during a subsequent fasting period(1,2) suggested the possibility that with the change in metabolic pattern, the activity of some enzymes involved in carbohydrate metabolism may also be altered. We considered particularly alkaline phosphatase because several authors had shown that the phosphatase activity of some tissues underwent changes due to starvation,

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protein depletion, and the feeding of different diets(3,4,5,6). Bellini and Cera(7) reported an increase of phosphatase activity in the rat intestine after the ingestion of neutral fats. This effect varied inversely with the chain length for butyric, lauric and stearic acids. Oleic acid was more active in increasing the activity than were the saturated fatty acids (8). It has also been shown that alkaline phosphatase activity in rat intestine decreases during fasting and increases during feeding. Furthermore, a meal containing fat causes a greater increase in lymph and plasma alkaline phosphatase activity than does a fat-free meal (9). The increase of alkaline phosphatase in the intestinal lymph following a fat containing meal does not occur in rats with biliary fistula or after ligation of the bile duct(10). Histochemical determinations of the acid and alkaline phosphatase activities of the small intestine of the rat have failed, however, to show any difference in activity after feeding high carbohydrate, high fat or high protein diets(11). In human subjects and in albino rats, serum alkaline phosphatase was lowered by fasting and raised by ingestion of fat. Proteins and carbohydrate had no effect(12).

In the following experiments liver phosphatase activity was studied during the absorptive and post-absorptive periods in rats adapted to a high fat diet, and in non-adapted rats fed a single high fat meal.

Materials and methods. Male adult Wistar rats were used throughout these experiments. They were housed in individual cages and fed *ad lib.* All diets were compounded so that casein supplied 15.5% of the total caloric content. These diets maintained satisfactory growth in all cases.

Composition of high carbohydrate diet: Casein 315 g, USP salt mixture #5 105 g, cane sugar 105 g, corn starch 1575 g, choline HCl 3 g, methyl linoleate 3 g, corn oil 15 g.

Composition of the high fat diet: Casein 315 g, USP salt mixture #5 96 g, cane sugar 60 g, butter fat 684 g, ruffex 60 g, choline HCl 3 g, methyl linoleate 3 g. corn oil 15 g. All diets were supplemented with vitamins to previously established levels (13). For determination of alkaline phosphatase activity, rats were sacrificed by means of a blow on the neck. The median lobe of the liver was removed, weighed, and homogenized in a Potter-Elvehjem homogenizer at high speed for 3 minutes. A 20% homogenate was made by diluting with ice cold distilled water. During the homogenizing period the tube was immersed in ice cold water. The homogenate was then filtered through a thin layer of surgical cotton in the cold room. The phosphatase activity was measured immediately. The Bessey, Lowry and Brock method(14) which uses p-nitrophenol phosphate† as a substrate was modified in order to avoid the turbidity interference due to the presence of insoluble particles. We therefore adapted the method for determining phosphatase activity in tissue homogenates as follows: For measuring alkaline phosphatase activity 1 ml of the glycine buffered substrate previously adjusted to pH 9.4 was pipeted into a test tube. It was kept for 5 minutes in a water bath at 38°C. An aliquot of liver homogenate (0.1-0.2 ml) was pipeted into the tube and the solution incubated at 38°C for thirty minutes. At the end of the incubation period the enzyme was inactivated by addition of 2 ml of a 10% solution of trichloroacetic acid. Sufficient water was then added to bring the final volume to 10.9 ml. The sample was filtered through Whatman #40 paper and 0.2 ml of 40% NaOH was then added to the filtrate. The color developed was read at a wavelength of 420 mμ with a Lumetron colorimeter. Acid phosphatase activity was measured by the same procedure at pH 4.8 using a citric acid buffer. This method, which eliminates the correction for turbidity, gave reproducible results throughout all the experiments. The activity was expressed in Bessey-Lowry units. A "B-L" unit or millimolar unit is the amount of phosphatase which will liberate 1 mM of p-nitrophenol per hour/1000 g of liver.

Results. Table I shows the effects of a 33-day adaptation to high fat and high carbohydrate diets. A group fed Rockland rat pellets, which is essentially a high carbohydrate diet (4.0% fat), is also included. The rats

† Supplied by Sigma Chemical Co., St. Louis, Mo.

TABLE I. Effect of Prolonged Feeding of a High Fat and High Carbohydrate Diet on Liver Phosphatase Activity during Absorption.

Group	No. rats	Diet	Adaptation period	B-L units alkaline phosphatase	B-L units acid phosphatase
I	5	Rockland rat pellets		14.0 ± .3	
II	7	High CHO	33 days	14.6 ± .9	498 ± 20
III	7	" fat	33 "	20.2 ± 2.7	517 ± 12

were sacrificed during the absorptive period, at which time food was still present in their stomachs and intestines.

Alkaline phosphatase activity was significantly increased for the high fat group, as compared to the other two. No difference was evident between the high carbohydrate and the Rockland fed animals.

No significant changes were noticed in the acid phosphatase activity for the high carbohydrate and high fat groups.

The effect of a 12-hour fast in animals which were previously fed a high fat and high carbohydrate diet for 30 days was investigated in a second group of experiments (Table II). No difference was observed in the liver alkaline phosphatase activity.

Finally Table III shows the phosphatase activity after a single meal of the high fat diet was consumed by animals previously fed the Rockland stock diet. The rats in this experiment were sacrificed during the absorptive period. As in experiment 1 the alkaline phosphatase activity was increased in comparison to the activity in controls fed the Rockland diet.

Discussion. The feeding of a high fat diet increased the alkaline phosphatase activity of rat liver during the absorption period. Acid phosphatase activity did not show any significant change. This increase of activity occurred only during absorption. Adaptation to high fat diets failed to produce any increase which would persist into the post-absorptive period. Thus it would appear that during fat absorption a transient increase of alkaline phosphatase activity occurs in the liver. The feeding of a single high fat meal increased the alkaline phosphatase activity of the liver during absorption in the same manner as did feeding a high fat diet over a considerable period of time.

These findings are in accord with those of other authors, who demonstrated that alkaline phosphatase activity of intestine and lymph increases during the feeding of fats.

TABLE II. Effect of Prolonged Feeding of High Fat and High Carbohydrate Diet on Liver Phosphatase Activity after 12 Hr Fasting.

Group	No. rats	Diet	Adaptation period	B-L units alkaline phosphatase
I	7	High CHO	30 days	14.5 ± .8
II	6	" fat	30 "	14.5 ± 3.4

TABLE III. Effect of a Single Meal of High Fat Diet on the Liver Alkaline Phosphatase Activity during Absorption.

Group	No. rats	Diet	B-L units alkaline phosphatase
I	8	High fat	23.3 ± 3.5
II	5	Rockland rat pellets	14.0 ± .3

Summary. 1. Feeding of a high fat diet increased the alkaline phosphatase activity of rat liver during the absorptive period. This activity returned to normal after a 12-hour fast. Adaptation to a high fat diet for 30 days did not alter this pattern. 2. These results indicate that the increase of alkaline phosphatase activity after feeding of fat is a transitory result of fat absorption and not a consequence of change of metabolic pattern due to fat adaptation. 3. The method using p-nitrophenol phosphate as a substrate was modified for determining phosphatase activity in tissue homogenates.

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Androgen and the *myo*-Inositol Content of Male Accessory Organs of the Rat.* (23492)

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The function of inositol in animal and plant physiology has been the subject of many investigations since its discovery more than one hundred years ago by Scherer(1). Inositol is required for growth of certain yeasts(2) and mice(3), and a dietary deficiency of this factor in hamsters is associated with reproductive disturbances(4). The importance of inositol as a lipotropic substance has been established(5). Furthermore, it has been demonstrated that *myo*-inositol is an essential growth factor for normal and malignant human cells in tissue culture(6,7). Mann(8) has reported the occurrence of relatively large quantities of free inositol in the seminal vesicles of the boar. Inositol was present to the extent of 2 to 3 g per 100 ml of seminal vesicle secretion.

The investigation presented here deals with the *myo*-inositol content of the male accessory organs of the rat during growth and the influence of androgen on the *myo*-inositol content of these organs. The significance of inositol in male accessory organs and secretions is obscure. It may be involved in the carbohydrate metabolism of these organs. Stetten and Stetten(9) demonstrated that deutero-labeled inositol is converted to glucose by the rat. Daughaday *et al.*(10) using glucose uniformly labeled with C^{14} showed that in the immature rat and chick embryo endogenous synthesis of inositol occurs.

Methods. Male rats of the Holtzman strain were used in this investigation and were fed Purina Laboratory Chow and raw carrots. The seminal vesicles, coagulating glands, dorsal and ventral prostates, and Cowper's glands were used in this study. Secretion-free organs were prepared by splitting glands lengthwise and removing the secretion by blotting on filter paper. Bilateral castration was performed on animals in which the effects of gonadectomy and androgen replacement were studied. A period of at least 20 days was allowed following castration for the regression of the male accessory organs. Testosterone propionate in oil† was given subcutaneously in daily doses of 500 μg. The selective uptake of *myo*-inositol by the seminal vesicles and dorsal prostates of castrate rats was studied. *Myo*-inositol was administered by stomach tube (25 mg per 100 g body wt) to fasting castrate and hormone-treated castrates 12, 8 and 4 hours before they were sacrificed. *Myo*-inositol was isolated and

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† Peranderen, Ciba Pharmaceutical Products, Summit, N. J.