bacilli grew more profusely in rats receiving glycerin, than in untreated ones. Since 3 of the saline treated pigs showed the enormous numbers of bacilli, glycerin cannot be the only factor. The extraordinary proliferation of bacilli in the lungs in contrast to that in the spleen and liver, and also the fact that the bacilli growing in the lung produce much less necrosis than those introduced intracutaneously, are perplexing phenomena. Further studies of these points and of the antibody titer in the various groups will be reported later.

Summary. When guinea pigs were infected with virulent human tubercle bacilli and prevented from becoming hypersensitive by daily subcutaneous injections of O.T., some developed pulmonary lesions teeming with bacilli. However, some of the control animals, demonstrated to be hypersensitive, showed identical lesions. It is, therefore, concluded that the development of lesions of this type is dependent on factors other than a lack of hypersensitivity.

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Utilization of β-hydroxybutyric Acid by Fed and Fasted Rats.*

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Whenever a substance or some experimental procedure produces a decrease in ketosis, it is of some importance to determine whether this effect is due to a decrease of ketone body production by the liver *(antiketogenesis)*, or to an increase in ketone body utilization by the muscles *(ketolysis)*. Particularly is this significant in considering the influence of glucose administration.[1]

In a series of recent studies, we could obtain no evidence that glucose can accelerate the rate of ketone utilization by the muscles of normal rabbits[2] or by the perfused dog heart.[3] However, the in-

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1 Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **118**, 290.

2 Mirsky, I. A., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **119**, 734.

3 Waters, E. T., Fletcher, J. P., and Mirsky, I. A., *Am. J. Physiol.*, 1938, **122**, 542.

vestigations of Butts and Deuel,[4] Butts,[5] Shapiro,[6] and others lead to the conclusion that glucose is ketolytic in action. In view of this discrepancy, it became of importance to reinvestigate the problem by another approach.

The method employed by Butts and Deuel, by Shapiro, and by some other workers studying the influence of various factors on ketosis, consists in measuring the urinary excretion of ketone bodies after the ingestion of a definite amount of either sodium acetoacetate or of sodium β-hydroxybutyrate. The difference between the amount fed and the amount excreted is interpreted to indicate the amount oxidized. Thus, when Butts and Deuel observed that rats maintained on a stock diet excreted minimal amounts of the fed ketone bodies as compared with fasted rats, or when Shapiro found that the administration of glucose or other glycogenic metabolites resulted in a decrease in the ketone excretion of rats fed diacetic acid, they concluded that glucose produces an increase in the oxidation of the ketone bodies.

In order to evaluate the significance of the above method and the findings obtained with it, we studied the actual disappearance of administered ketone bodies from the entire body of nephrectomized rats. Five sets of female rats were employed (Table I). Two sets (B and E) consisted of animals on a stock diet until a few minutes before the experiment was started. The other 3 sets consisted of animals previously fasted for 24 hours. A bilateral nephrectomy was performed on all the animals under ether anesthesia. Immediately after the completion of the operation, the fed animals (B and E) received an intraperitoneal injection of 100 mg glucose per 100 g of body weight, while one set of fasted animals (C) received a similar injection of glucose, and the other 2 (A and D) an injection of saline. After allowing the animals to recover from the anesthesia, a definite amount of racemic sodium β-hydroxybutyrate per unit of body weight was injected intravenously into one set of fed rats receiving glucose intraperitoneally (E), and one set of fasted rats receiving saline (D), the remaining groups being used for control purposes. A 40-minute period was permitted to elapse before the animals were sacrificed and reweighed. They were then ground up repeatedly in an ordinary meat grinder, and mixed thoroughly to ensure a good mixture. The β-hydroxybutyric acid

[4] Butts, J. S., and Deuel, H. J., *J. Biol. Chem.*, 1933, **100**, 415.
[5] Butts, J. S., *J. Biol. Chem.*, 1934, **105**, 87.
[6] Shapiro, I., *J. Biol. Chem.*, 1935, **108**, 373.

TABLE I.

Utilization of Intravenously Injected β-hydroxybutyric Acid by Nephrectomized Fed and Fasted Rats in a 40-Minute Period.

Experimental Procedure	No. of Rats	Body wt, g	Surface area, cm^2	β-hydroxybutyrate injected, m M/K	β-hydroxybutyric acid recovered, m M/K	β-hydroxybutyric acid utilization		
						Actual data, m M/K	Corrected for initial content, m M/K	Corrected for initial content, m M/sq M/hr
A. Fasted-Saline Treated Control	20	143.5 ± 4.7	246.5 ± 4.5	0	0.58 ± 0.065	—	—	—
B. Fed-Glucose Treated Control	10	157.0 ± 4.7	260.0 ± 4.6	0	0.21 ± 0.015	—	—	—
C. Fasted-Glucose Treated Control	10	138.5 ± 3.0	241.5 ± 3.1	0	0.20 ± 0.014	—	—	—
D. Fasted-Saline Treated	20	146.0 ± 3.9	249.0 ± 3.9	8.20 ± 0.030	3.99 ± 0.105	4.21 ± 0.105	4.79 ± 0.124	28.1
E. Fed-Glucose Treated	20	148.0 ± 2.9	251.0 ± 2.9	8.40 ± 0.044	3.49 ± 0.116	4.91 ± 0.116	5.12 ± 0.116	30.1

Deviation from mean = ± standard error of mean.

$$\frac{\text{Difference of mean E — D}}{\text{Standard error of means}} = \frac{4.5 \text{ (uncorrected)}}{1.9 \text{ (corrected)}}$$

content of 3 aliquots of each ground-up rat was then determined by a modification of Barnes' method.[7]

Recovery experiments were performed on fed rats sacrificed immediately after the injection of β-hydroxybutyric acid, to determine the efficacy of all the various steps involved. The data thus obtained reveal that the procedure is very reliable since an average recovery of 99.6% was obtained in 5 rats. The details of the experimental procedures will be reported in a subsequent paper.

The data summarized in Table I reveal that if no correction is made for the initial β-hydroxybutyric acid content, a small, though perhaps statistically reliable difference exists between the utilization of β-hydroxybutyric acid by fed rats receiving glucose as compared with fasting rats receiving saline. However, when the spontaneous ketogenesis of fed and fasted rats is taken into account by adding the initial ketone content of the animals to the amount administered, the difference between the fed and fasted rats loses its statistical reliability. Therefore, it is obvious that glucose (or feeding) does not influence the actual utilization of β-hydroxybutyric acid. It is probable from this and other studies still in progress, that the results of Butts and Deuel and of Shapiro must be due to some factor other than ketolysis. The fact that the spontaneous ketogenesis of fasted rats can be decreased by glucose administration suggests the probability that the apparent ketolytic activity reported by the above investigators is due to a cessation of this spontaneous or induced ketogenesis *(antiketogenesis)*.

Summary. There is no significant difference in the rate of utilization of administered β-hydroxybutyric acid by fed or fasted rats receiving glucose. The spontaneous ketogenesis of fasted rats is inhibited by glucose administration and may account for the apparent ketolytic activity reported by others.

[7] Barnes, R. H., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 352.