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# EFFECT OF THE THYROID ON BUTYRATE OXIDATION IN THE RAT HEART*

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**Abstract**—Oxidation of butyrate by rat heart homogenates in the presence of malate, nicotinamide or endogenous "sparkers" has been studied. It was found that heart homogenates from hyperthyroid rats oxidized butyrate at a higher rate than those from normal or hypothyroid animals. It is suggested that these findings may have some bearings on the mechanisms of cardiac abnormalities associated with thyroid dysfunction.

IT HAS been recognized for many years that the thyroid has a profound effect on cardiac function. The hyperthyroid individual exhibits tachycardia and an increased cardiac output[1] terminating eventually in high output failure.[2] In hypothyroidism the heart is subject to low output failure.[3] The causes of these effects are unknown. In recent years much work has been done in the area of *in vitro* metabolism of the heart in search of a possible explanation for the *in vivo* manifestations of altered thyroid function. The work of Gemmill,[4] Ullrick and Whitehorn[5] and Barker[6] are examples. The demonstration by Bing *et al.*[7] and Gordon[8] that the heart normally utilizes fatty acids for a large portion of its energy requirements has focused our attention on the oxidation of fatty acids in the heart as a possible point of attack on the problem of relating thyroid disease to altered cardiac function.

Olsen and Piatnek[9], employing cardiac catheterization, have shown that the hearts of thyrotoxic dogs extracts a greater amount of fatty acids from the blood than do the hearts of euthyroid animals. It appeared to be possible that the ability of the thyrotoxic heart to use fatty acids in excess would support the increased cardiac work. Conversely, if the hypothyroid heart oxidized fatty acids in amounts less than normal, a partial explanation of low output failure in hypothyroidism would be possible.

## EXPERIMENTAL

Young rats (130–150 g) of the Denver strain were used. They were made hyperthyroid by injection of 200 $\mu$g of Na-L-thyroxine pentahydrate‡ per g, subcutaneously, once daily for two weeks. Hypothyroidism was induced by surgical thyroidectomy, injection of $I^{131}$ intraperitoneally, or by administration *ad libitum* of 0·1 per cent thiouracil in the drinking water. Surgically thyroidectomized animals were provided with 1 per cent calcium lactate in the drinking water for one week and sacrificed 14 days postoperatively. $I^{131}$ was injected in a dose of one mc per rat. These animals were used approximately 30 days after injection. Thiouracil was given in the drinking

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water for 14 days prior to experimentation. The basic metabolic rate of the animals was determined by a modification of the method of d'Amour and Blood[10]. Conventional manometric techniques were used to determine butyrate oxidation. The animal was stunned by a blow on the head, the heart immediately removed and placed in ice-cold 0·25 M sucrose. Only the ventricles and intraventricular septum were used. The tissues were blotted dry, weighed and placed in a Tenbrock glass homogenizer containing enough ice-cold homogenizing fluid to yield 80 mg of tissue per ml. The homogenizing medium consisted of 80 parts of 0·25 M sucrose and 20 parts of 0·05 M potassium phosphate buffer, pH 7·4. The heart was rapidly homogenized by a combination of hand and motor driven homogenization with a loose-fitting pestle. The homogenate was strained through two layers of gauze to remove any pieces of connective tissue. Of the homogenate, 0·5 ml was pipetted into ice-cold Warburg flasks containing the reaction mixture described by Plaut and Plaut,[11] except that in some flasks 0·5 $\mu$mole of malate was replaced by 15·0 $\mu$mole of nicotinamide or water. Butyrate was present in a final concentration of 0·001 M (1·5 $\mu$mole per flask). The final volume was 1·5 ml in the main compartment and the center well contained 0·1 ml of 10 per cent KOH. All solutions except nicotinamide were adjusted to pH 7·4 with KOH. The flasks were placed in the water bath at 30 °C, and, after an equilibration period of 10 min the stopcocks were closed and readings taken every 10 min for one hr. A final reading was taken at 90 min. In all experiments, the homogenate was ice-cold during preparation and no more than 15 min elapsed between killing the animal and placing the flasks in the water bath. Deviation from this procedure resulted in inactive preparations.

## RESULTS AND DISCUSSION

All results are corrected for control flasks containing no butyrate and are expressed as $\mu$l. of oxygen uptake per 40 mg of heart tissue, wet weight. The control flasks containing no butyrate usually had an oxygen uptake of about one-half that obtained when butyrate was added. When duplicate flasks were employed, the values were averaged and treated as one value for the statistical analysis.

TABLE 1. $O_2$ UPTAKE IN $mm^3$ DUE TO ADDED BUTYRATE BY 40 mg OF HOMOGENIZED RAT HEART VENTRICLES

| | | Additions to Flasks | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Butyrate | | | | Malate Plus Butyrate | | | | Nicotinamide Plus Butyrate | | | |
| Group | BMR* | 40 min | S.E. | 90 min | S.E. | 40 min | S.E. | 90 min | S.E. | 40 min | S.E. | 90 min | S.E. |
| Control | 2·67 | 15·0† (11) | ±2·36 | 21·6 (11) | ±2·88 | 15·7 (16) | ±2·51 | 27·4 (16) | ±3·73 | 49·0 (21) | ±4·10 | 65·3 (21) | ±4·13 |
| Hyper-thyroid | 3·47‡ | 76·3 (12) | ±1·92 | 112·2‡ (12) | ±2·89 | 91·0‡ (14) | ±1·96 | 131·4‡ (14) | ±2·35 | 70·6‡ (14) | ±3·40 | 123·2‡ (14) | ±5·86 |
| $I^{131}$ Treated | 1·73‡ | 7·1 (14) | ±1·50 | 10·2‡ (14) | ±1·94 | 9·9 (15) | ±1·70 | 16·9 (15) | ±2·40 | 24·3‡ (16) | ±3·80 | 43·9 (16) | ±7·30 |
| Thyroid-ectomized | 1·60‡ | | | | | 9·3 (16) | ±2·14 | 11·9‡ (16) | ±3·01 | 12·5‡ (16 | ±2·82 | 18·5‡ (16 | ±3·73 |
| Thio-uracil treated | 1·86‡ | 7·9 (4) | ±1·38 | 10·5 (4) | ±4·19 | 7·8 (9) | ±1·49 | 12·3‡ (9) | ±2·09 | 22·3‡ (9) | ±2·81 | 30·6‡ (9) | ±2·96 |

* Basal metabolic rate, ml of oxygen consumed at standard conditions/min per 100 g of rat.
† Numbers in parenthesis indicate the number of animals used.
‡ Significantly different from control, $p < 0·001$.

As can be seen from Table 1, oxygen uptake due to added butyrate in heart homogenates obtained from euthyroid animals was considerably increased by addition of nicotinamide. Presumably the increased oxygen uptake in the presence of nicotinamide is attributable to maintenance of functional diphosphopyridine nucleotide (DPN) since this is a well-known effect of nicotinamide.[12] Functional DPN then would be a limiting factor in the systems not containing nicotinamide. Addition of DPN to normal heart homogenates results in greatly increased total oxygen uptake, very little of which could be ascribed to oxidation of butyrate. Similar results are reported by Allen *et al.*,[13] except that these investigators observed increased $C^{14}O_2$-production from $C^{14}$-labelled butyrate when DPN was added. This failure of added DPN to stimulate the oxygen uptake due to butyrate illustrates the importance of binding of DPN.[14, 15] Addition of malate to heart homogenates from euthyroid animals resulted in very little increase in oxygen uptake due to butyrate. This is explained by the probability that there are enough oxaloacetate or oxaloacetate precursors present endogenously to "spark" the oxidation of butyrate.

Oxygen uptake due to butyrate was diminished in the heart homogenates obtained from hypothyroid animals in all systems employed. The reduction of the butyrate oxidation in the nicotinamide-protected system was the most consistent finding. Since one possibility is that nicotinamide is acting by protecting functional DPN, these observations may indicate a decreased amount of functional DPN in these homogenates. Because the amounts of coenzyme A,[16] cytochrome-*c*[17] and phosphate acceptors,[18] are decreased in hypothyroid tissues, these substances were added to the flasks in various experiments in attempts to raise the lowered values toward normal. No increase in oxygen uptake due to butyrate was observed when any one of these substances was added. In addition, flavine adenine dinucleotide, lipoic acid and L-triiodothyronine were added to heart homogenates from hypothyroid animals in separate experiments without any increase in butyrate oxidation.

In heart homogenates obtained from hyperthyroid animals, the oxygen uptake due to butyrate was increased to a marked degree. It is interesting that the oxidation in the nicotinamide-protected system was no greater than that measured in the malate and endogenous systems; therefore, it is possible that functional DPN is not rate-limiting in the heart homogenate from hyperthyroid animals. Very little information is available in the literature concerning the amounts of functional DPN in heart tissue obtained from hypothyroid or hyperthyroid animals. There are several reports of decreased total pyridine nucleotide concentrations in livers of hyperthyroid animals.[18, 19] It is evident from our results that the total pyridine nucleotide may not be the important consideration, but rather that the amount of functional DPN may be the crucial factor.

If the increased ability to oxidize butyrate, shown by the hyperthyroid heart in our experiments, were to extend to longer chain fatty acids, it is possible that the hyperthyroid heart would be able, on this basis, to support a significantly increased work load. The hyperthyroid heart, less able to oxidize fat, would not support a large increase in work load and would be subject to low output failure.

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