is somewhat slower following irradiation at the level used in this study.

Summary. Alterations in serum and urine tyrosine levels were observed in starved irradiated rats and in starved control rats. It appears that protein catabolism is somewhat slower following irradiation at the 800 r level we used.

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Influence of Oxidation Products of Epinephrine upon Adenosinetriphosphatase Activity of Uterine Muscle Preparation.* (23817)

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The physiological response of uterine muscle to epinephrine has been extensively studied, but biochemical aspects of the epinephrine effect have not been elucidated. The typical response of the non-pregnant uterus to epinephrine in most species is diphasic with an initial contractile response followed by a longer period of inhibition(1,2). The diphasic response is not typical during pregnancy(1). An attempt has been made to gain information concerning the biochemical changes accompanying hormonal stimulation of uterine contractility. It was found that the dephosphorylation of adenosinetriphosphate (ATP) by a contractile protein preparation from bovine uterine muscle was inhibited by oxidation products of epinephrine. Although the enzymatic activity is referred to here as adenosinetriphosphatase (ATPase) activity, it is probable that more than one enzyme is involved.

Materials and methods. The procedure commonly used for preparation of structural elements from skeletal muscle(3,4) was modified for isolation of the contractile elements of uterine muscle. Uterine tissue was obtained from 4 non-pregnant cows which, from examination of ovaries and luminal fluids, were apparently not in estrus at time of slaughter. The endometrium was carefully separated and discarded. The myometrium from the 4 animals was pooled and then chopped in a chilled meat grinder. The extraction and purification were carried out at 5°C. Glass-distilled water was used. One hundred and five grams of tissue were extracted with 350 ml of a solution 0.3 *M* in KCl, 0.15 *M* in K_2HPO_4 and 0.15 *M* in KH_2PO_4 by constant agitation with an electric stirrer for 5½ hours. The mixture was diluted with 1.4 liters of water and filtered through cheese cloth. The filtrate was diluted with water to 4.2 liters. Precipitation of protein was complete in about 3 days. Most of the supernatant was siphoned off and the rest removed by centrifugation. The precipitate was dissolved in 2 *M* KCl. The solution was then diluted to KCl concentration of 0.44 *M* and centrifuged to remove any precipitated material. The supernatant was filtered through glass wool, and diluted with water to KCl concentration of 0.22 *M*, which resulted in precipitation of the desired material. Pre-

* This work was initiated while the senior author was a graduate student in Department of Dairy Science at University of Illinois and has been continued under the auspices of the U. S. Atomic Energy Commission.

cipitate was collected by centrifugation and dissolved in 2 *M* KCl. Purification procedure was repeated 3 more times. Within the range of ultraviolet light from 260 mμ to 290 mμ, this preparation showed a maximum absorption at 260 mμ with a 260 mμ 280 mμ ratio of 1.34. This ratio is explained by the presence of nucleic acid(6.7). The nature of the constituent proteins and nucleoproteins is discussed in more detail by Snellman and Tenow (5), who studied preparations similar to the one used here. *ATPase* was assayed at 38°C by measurement of rate of inorganic phosphate appearance under anaerobic conditions in $NaHCO_3$ buffer at pH 7.3 in the presence of added ATP. Warburg flasks were used with a gas phase of 95% N_2, 5% CO_2 mixture. Results represent calculations upon total phosphate released enzymatically. Inorganic phosphate was determined by the method of Fiske and SubbaRow(8); 4% trichloroacetic acid was used as protein precipitant. *Oxidation product* or products of epinephrine were estimated as adrenochrome. Measurements of optical density of reaction mixtures in one group of experiments and of partially oxidized epinephrine solutions in a second group were used to estimate concentration of oxidation products of epinephrine. Measurements made using the Klett-Summerson photoelectric colorimeter with No. 50 filter were compared to a standard adrenochrome assay(9). No further attempt was made to identify the oxidation products for these experiments.

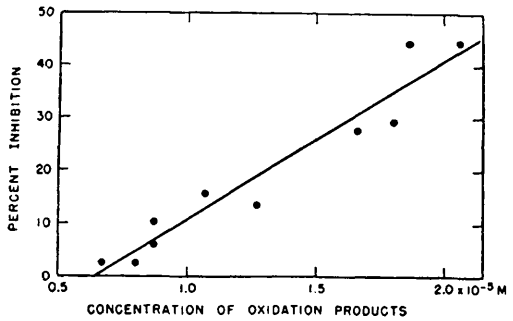

FIG. 1. Relationship between molar concentration of oxidation products of epinephrine, estimated as adrenochrome, and inhibition of ATPase activity. Reaction mixtures contained 37.5 μg N/ml, 0.0260 *M* $NaHCO_3$, 0.5 *M* KCl, and 0.005 *M* ATP. Regression and associated stand. error for relationship between percent inhibition of ATPase activity and concentration of oxidation products was 30.26 ± 2.89. Stand. dev. from regression was 4.43. Regression was highly significant ($p = < 0.001$).

Results. In the first group of experiments the uterine preparation was incubated with epinephrine (6×10^{-5} *M*) under aerobic conditions for periods of time ranging from ¼ to 30 minutes before gassing with 95% N_2-5% CO_2. As aerobic incubation period increased there was a progressive increase in epinephrine oxidation. After 20 minutes of anaerobic gassing the assay was begun by addition of ATP from the side arm. Estimations of adrenochrome concentration were made at the end of the assay period. The average ATPase of the controls was 0.70 μM of phosphate liberated from ATP per ml reaction mixture (37.5 μg N) per hour. Fig. 1 presents the relationship found between oxidation products of epinephrine and inhibition of ATPase activity.

In another group of experiments, solutions of epinephrine were allowed to autoxidize in bicarbonate buffer; oxidation was terminated by anaerobic gassing. Adrenochrome concentration was determined immediately prior to use. The solutions were kept anaerobic until they were used; aliquots were transferred to a side arm of double-side arm Warburg flasks (the other side arm contained ATP), which were immediately gassed with a 95% N_2, 5% CO_2 mixture. The total aerobic exposure of the solutions, which were initially anaerobic, was not more than 3 minutes. Little if any increase in oxidation of epinephrine resulted after the evaluation in the colorimeter. The flasks were anaerobically gassed for 20 minutes. First, epinephrine solutions were tipped; ATP was tipped in 12 minutes later, and the ATPase activity was estimated as before. A 4.4×10^{-6} *M* concentration of autoxidation products estimated as adrenochrome caused a 27% inhibition of ATPase activity, 1.5×10^{-6} *M* caused a 15% inhibition, and detectable effects were observed in concentrations between 10^{-6} *M* to 10^{-7} *M*. These lowest concentrations were not estimated with exactness. The original epinephrine concentration was 6×10^{-5} *M* for most of these assays. However, for one group of assays, 6×10^{-6} *M* epinephrine was used. No inhibi-

tion of ATPase activity appeared attributable to epinephrine itself.

Discussion. Dickens and Glock(10) found 20% inhibition of ATPase activity of myosin from rat skeletal muscle with concentrations of adrenochrome of 10^{-4} *M*. We observed a similar inhibition with concentrations of epinephrine oxidation products of 1.3 x 10^{-5} *M* and 3 x 10^{-6} *M*. Detectable effects were observed even at concentrations below 10^{-6} *M*. Adrenochrome, as in the case of other quinones, has been found to oxidize reversibly the –SH groups of several enzymes(11). Dickens and Glock(10) concluded that adrenochrome oxidatively inhibited a rat skeletal muscle myosin preparation and Bailey and Perry(12) have shown that oxidative removal of the –SH groups of myosin inhibited both ATPase activity and ability to combine with actin to form actomyosin. The inhibition observed in these present studies is perhaps related to an action upon –SH groups. The question may be raised whether the prolonged inhibition of uterine motility which is observed following epinephrine treatment *in vivo* and *in vitro*(1) might be related to the enzymatic inhibition observed in these present studies. The possibility of epinephrine oxidation *in vivo* is supported by the work of Wajzer(13), which suggested the existence of an epinephrine-adrenochrome system in mammalian skeletal muscle. Also, Green and Richter(14) have shown that the oxidation of epinephrine *in vitro* is catalyzed by a cyanide-insensitive enzymatic system present in heart and skeletal muscle and by the cytochrome-indophenol oxidase system of heart muscle. However, Schayer and Smiley(15), using isotope technics, concluded that adrenochrome is probably not an intermediate in the metabolism of epinephrine. Nevertheless, their results did not rule out the possibility that in its metabolism, epinephrine might become attached to an enzyme where it is oxidized to adrenochrome, and is then metabolized still further without being released(15). Although it does not seem possible to reconcile the question at this point, the present study suggests that oxidation products of epinephrine, if present, might be expected to have a significant physiological role.

Summary. Oxidation products of epinephrine, estimated as adrenochrome, inhibited ATPase activity of a uterine muscle preparation. The effects were observed with amounts approaching physiological concentrations (10^{-6} *M* to 10^{-7} *M*). No inhibition attributable to epinephrine alone was observed in the range of epinephrine concentrations studied (6 x 10^{-5} to 6 x 10^{-6} *M*).

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