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A Study of Stimulated Respiration in the Adrenal Gland

Owen Foster Kline

Kline — Respiration in the Adrenal Gland

A STUDY OF A STIMULATED RESPIRATION IN THE ADRENAL GLAND
I. INTRODUCTORY CONSIDERATIONS

A Thesis
Presented to
the Faculty of the Department of Biology
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In Partial Fulfillment of
the Requirements for the Degree
Master of Science

by
Owen Foster Kline Jr.
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ACKNOWLEDGEMENT

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INTRODUCTION

The purpose of this paper is to present certain preliminary observations on what is believed to be a completely new special metabolism in the adrenal gland of Hereford cattle. Although no direct correlation between any of the substances isolable from the adrenal gland and the respiratory system to be described in this paper has been obtained, it is nevertheless necessary to present a review of the chemical and medical data available pertaining to these glands.

The mammalian adrenal gland consists of two parts which are closely related anatomically, have separate origins, and so far as is known, function independently of each other. The central portion of the gland is known as the medulla, while the outer enveloping tissue is called the cortex.

According to Keene and Hewer (1927) the medulla is derived from primitive cell masses which have separated from the neural crest. Migrating from this presumptive area, some of the cells of mes-ectodermal tissue undergo differentiation into chromaffin tissue. These cells come to lie on either side of the midline in the abdomen and become enveloped with cortical tissue to constitute the adrenal medulla. The cortex is derived from mesodermal tissue and arises as a bud from the coelomic epithelium

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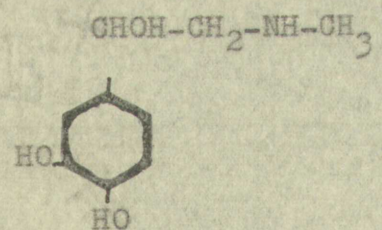
covering the inner side of the fore part of the mesnephros. The coelomic epithelium immediately behind this area gives rise to the germinal epithelium from which the sex glands develop. However the description of the development of these glands in some embryology texts (Arey, 1947), outlines this process differently. "The chromaffin cells of the medulla are descended from the primitive ganglia of the coeliac plexus of the sympathetic system. In embryos of seven weeks, when the cortex is already prominent, masses of these cells begin to invade the medial side of the cortical primordium. The continued migration of these cell clusters brings them to a central position in the gland."

The medulla is composed of closely packed groups of polyhedral cells which contain chromaffin granules. These granules are considered the site of medullary secretion.

The cells of the cortex are arranged in three zones, from the outside inwards: (1) zona glomerulosa, in which the cells are arranged in a circular or oval pattern, (2) zona fasciculata, in which the cells are grouped in columns, and (3) zona reticularis, in which the cells are formed in a network of cell cords. The cells of the cortex contain a doubly refractive lipid material which may be the active cortical hormone or its precursor (Keene and Hewer, 1927).

The adrenal gland is a very vascular organ and receives six to seven cc. of blood per gram of tissue per minute (Neuman, 1912). It is supplied by three small arteries, which respectively originate from the inferior phrenic artery, the renal artery and the aorta. These arteries form plexuses in the cortex which are continuous with the sinuses of the medulla and drain into a central vein of the latter. The vein from the right adrenal empties into the inferior vena cava and the vein from the left into the renal vein.

The active principle of the adrenal medulla was isolated in pure form in 1901 by Takamine and Aldrich and has the empirical formula, $C_9H_{13}O_3N$. It has been given various names such as adrenaline (adrenalin), epinephrine, adrenin and suprarenin. Adrenaline is the most commonly used. The structural formula is as follows:



Adrenaline has a variety of physiological actions. (1) A differential action occurs upon the blood vessels when amounts within the physiological range (about 0.01 cc. per kilogram of 1 in 10,000 solution administered subcutaneously). The arterioles and capillaries of the skin,

mucous membranes and splanchnic viscera (except the intestinal vessels which usually dilate) are constricted; the vessels of the muscles at the same time dilate. With such a dose the constrictor effect overshadows the dilator effect, the net result being a rise in blood pressure. (2) Larger doses cause pulmonary vasoconstriction. The arterioles in the brain are passively dilated by the rise in blood pressure. (3) In very small amounts (0.01 cc per kilogram of 1 in 50,000 or 1 in 100,000 solution) a fall in blood pressure results. This is attributed to the fact that the dilation of the muscles overshadows the constrictor effect of the arterioles and capillaries of the skin, mucous membranes and splanchnic viscera (Hartman _et_. _al._, 1928).

Adrenaline inhibits the muscles of the stomach, intestine, bronchioles and walls of the urinary bladder. Both the tone and movements of the intestine are inhibited; the bronchioles are dilated. It excites the muscles of the gall bladder, ureter, trigone and sphincter of the bladder, the retractor and the pyloric, ileocoelic and internal anal sphincters.

The administration of adrenaline affects carbohydrate metabolism (Britton and Silvetter, 1932). It causes hyperclycemia and glycosuria. It shows an antagonism to insulin, thus relieving hypoglycemic convulsions. This is due to the mobilization of sugar from liver glycogen, a storage form carbohydrate. The glycogen of the muscles

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tinal vessels [illegible] (dilate) [illegible]
vessels of the muscles [illegible]. [illegible] such
a dose the [illegible] the dilator
effect, the net result [illegible] in blood pressure.
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in blood pressure. (3) [illegible] per
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storage form carbohydrate. The [illegible]

is also decreased by adrenaline. In animals which have been on a prolonged fast or under conditions which have depleted the liver glycogen stores, adrenaline causes an increase in liver glycogen. The latter is due to the breakdown of muscle glycogen to lactic acid which, diffusing into the blood, is carried to the liver where resynthesis to glycogen occurs (Cori and Cori, 1928). There is a fall in inorganic phosphate of the blood apparently due to the phosphorylation of glycogen and the formation of glucose monophosphate.

Adrenaline also affects the general metabolism of the body (Soskin, 1927). It causes an increased oxygen consumption of 20 to 40 per cent and carbon dioxide production is increased by 30 to 50 per cent. There is an increase in basal metabolic rate and the temperature of the muscles rises.

The dose of adrenaline required to produce a detectable metabolic response (rise in blood sugar and in metabolic rate) is considerably less than that which will cause a rise in blood pressure. Due to the rapid inactivation of adrenaline in the body, a given dose has a much greater effect upon metabolism if administered continuously over a period than if given in a single injection (Rogoff, 1935).

The adrenal medulla is not essential to life. In animals one adrenal may be removed completely and the

is also decreased by adrenaline. [illegible]

been on a prolonged [illegible]

depleted the liver glycogen [illegible]

increase in liver glycogen [illegible]

breakdown of muscle glycogen [illegible]

into the blood, [illegible]

to glycogen occurs (Cori and Cori, 1930). [illegible]

in inorganic phosphate of the blood [illegible]

phosphorylation of glycogen [illegible]

monophosphate.

Adrenaline also [illegible]

the body (Soskin, 1927). [illegible]

consumption of 20 to 40 per cent [illegible]

duction is increased by 30 to [illegible]

increase in basal metabolic rate [illegible]

the muscles [illegible].

The case of [illegible]

detectable metabolic [illegible]

metabolic rate) [illegible]

cause a rise in blood pressure. [illegible]

ation of adrenaline [illegible]

greater effect upon metabolism [illegible]

over a period than [illegible]

1935).

The adrenal [illegible]

animals [illegible]

medulla of the other excised without any apparent ill effect. It has been suggested that the medulla liberates its secretion in significant amounts only under conditions which call for unusual effort on the part of the body to perform work, to prevent changes in its internal environment or to resist threatened dangers. In such times of stress the medullary secretion, it is believed, reinforces the sympathetic nervous system. This adrenaline nervous cooperation enables the several bodily reactions under their influence to act at maximal efficiency (Cannon and de la Paz, 1931).

The adrenal cortex, unlike the adrenal medulla, is necessary for life. Removal of more than five-sixths of this portion of the adrenal causes death within a few days. Rogoff and Stewart (1928) showed that the life of completely adrenalectomized animals could be prolonged by the injection of a cortical extract combined with the transfusion of saline. Hartman _et_. _al_. (1928) called this extract cortin.

About twenty crystalline steriod compounds which exhibit in greater or less degree the physiological properties of crude cortical extracts have been isolated from the adrenal cortex. The following are the formulae of some of these physiologically active substances.

is also decreased by adrenaline. [illegible]

been on a prolonged [illegible]

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detectable metabolic [illegible]

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greater effect upon metabolism [illegible]

over a period than [illegible]

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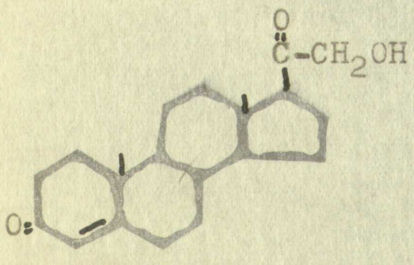
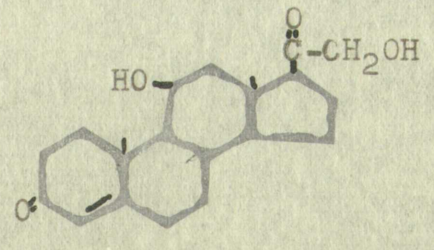
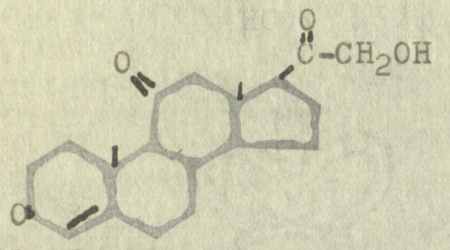
animals [illegible]



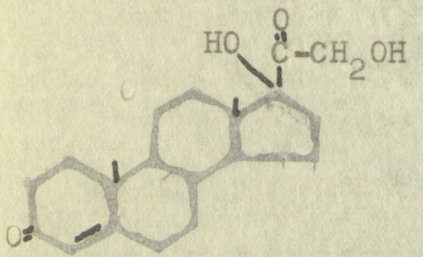
DESOXYCORTICOSTERONE



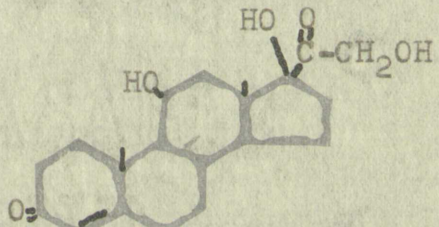
CORTICOSTERONE



11-DEHYDROCORTICOSTERONE



17-HYDROXYDESOXYCORTICOSTERONE

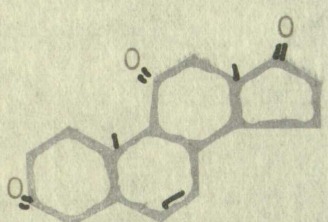


17-HYDROXYCORTICOSTERONE

A material called the amorphous fraction remains after the removal of the crystalline compounds from the crude extract.

In addition to the above compounds, steroids with estrogenic and androgenic activity (the 17-keto steroids) have been isolated from the gland. They are estrone, progesterone, 17- β progesterone, adrenosterone, androstenedione-3-17 and androstenediol-3- β -one-17.

The androgen, adrenosterone, is present in largest amounts. Its formula is:



ADRENOSTERONE

A completely adrenalectomized animal shows the following symptoms during its short survival period: loss of appetite (particularly for fats), vomiting, diarrhea, rapid loss of weight, weakness and prostration, fall in body temperature by several degrees, and a reduction of 20 per cent or so in the basal metabolic rate (Banting and Gairns, 1926). The blood becomes concentrated (loss of plasma water), shows a fall in sodium and sugar, and a rise in non-protein nitrogen and other signs of renal failure (Swingle et al., 1933). The glycogen stores in the liver and muscles are decreased. The above symptoms may be prevented by the administration of an extract of the adrenal cortex (Rogoff and Stewart, 1928).

The adrenal cortex is concerned with water metabolism and with the metabolism of sodium and potassium (Swingle et al., 1934). In adrenalectomized animals there is a marked reduction in total base which is due to the loss of sodium. The potassium concentration is raised above the normal lëvel, the rise being due to reduced excretion by

The androgen, adrenosterone [illegible]
the formula is:

ADRENOSTERONE

A completely adrenalectomized [illegible]
following symptoms [illegible] loss
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rapid loss of weight, [illegible]
body temperature [illegible]
20 per cent or more [illegible]
[illegible], 19[illegible]. The blood [illegible]
plasma water), [illegible]
in non-protein [illegible]
([illegible] et al., 19[illegible]). [illegible]
and [illegible].
prevented by [illegible]
cortex ([illegible]).

The adrenal cortex [illegible]
and with the metabolism of protein [illegible]
et al., 1944). In adrenalectomized [illegible]
marked reduction in [illegible]
of sodium. The [illegible]
normal level, the [illegible]

the kidney and leakage from the tissue cells into the extracellular fluid. The loss of sodium is due to the diminished reabsorption of sodium by the renal tubules and is accompanied by an increased excretion of water (Harrison and Darrow, 1939). Thus dehydration results. Therefore, the administration of sodium chloride to adrenalectomized animals and a reduction in the potassium intake gives a definitely beneficial result. The sodium rather than the chloride ion is the important factor (Swingle et al., 1934).

Carbohydrate metabolism is affected by those factors of the cortex which have an oxygen atom at carbon eleven. The fundamental action of these compounds is upon the conversion of protein to glucose, the hyperglycemic condition being accompanied by an increase in glycogen stores. Adrenal steroid diabetes has been demonstrated in normal rats which were forced to ingest a diet rich in carbohydrates but of normal caloric value (Britton and Silvette, 1931, 34, 38). This adrenal diabetes is highly resistant to insulin. Corticosterone, 17-hydroxycorticosterone and 17-hydroxy-11-dehydrocorticosterone produce hyperglycemia and glucosuria in normal rats. These signs of diabetes have been induced in normal force-fed rats by the administration of pure adrenocorticotropic hormone.

The mechanism by means of which the adrenal extracts

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The reduction by [illegible] [illegible] [illegible] [illegible] adrenal extracts

produce glucosuria is in part by stimulating gluconeogenesis, but as the increase in nitrogen excretion is insufficient to account for the extent of glucosuria, interference with glucose oxidation is also postulated (Ingle et. al., 1946).

Since cortical extracts postpone muscle fatigue, a normal animal is capable of greater work under its influence. The action is exhibited to the greatest degree by those compounds with an oxygen at the eleven carbon.

Corticosterone, dehydrocorticosterone and 11-dehydro-17-hydroxy corticosterone (compound E) exert a minimal effect upon water and salt metabolism (Thorn et. al., 1941). The restoration of normal kidney function is obtained by desoxycorticosterone administration but to a greater degree by the amorphous fraction. The most outstanding action of desoxycorticosterone is upon salt and water metabolism; it increases the plasma volume and the concentration of sodium in the body fluid, but reduces that of potassium (Miller and Darrow, 1941). As a result of its effect in causing the retention of sodium and water, desoxycorticosterone administration may be followed by an edema. Larger doses of desoxycorticosterone may have a serious effect, such as hypertension, dilatation of the right ventricle and pulmonary congestion. Death may result from its unwise clinical use. Addition of potassium salts or a

produce glucosuria is in part by stimulating gluconeo-genesis, but as the increase in glycogen deposition is insufficient to account for the extent of [illegible] interference with glucose oxidation [illegible] (Ingle et. al., 1946).

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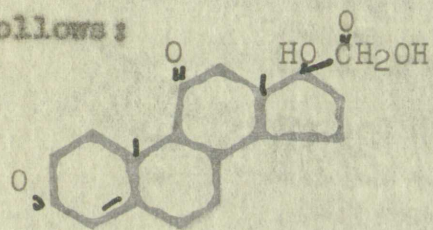
reduction in sodium intake will benefit patients suffering from an overdose of desoxycorticosterone (Kuhlman et al., 1939).

A recent development of great promise in medicine is the discovery of the value of 11-dehydro-17-hydroxy-corticosterone in the treatment of rheumatoid arthritis and acute rheumatism (Hench et al., 1949). This compound is better known as Kendall's Compound E or cortisone. The formula is as follows:



COMPOUND E

The mode of action of compound E is not known. However, in view of the large doses (up to ten milligrams daily) it is unlikely that it merely substitutes for a deficiency of the subject's cortical hormone. Permanent damage to the adrenal cortex may result from the continued administration of compound E.

Several observations point to the cortex as being in some way associated with the gonads. Among the observations are: (a) animals during heat or pregnancy or injected with the luteinizing principle of the hypophysis withstand adrenalectomy better than at other times, and progesterone

lengthens the life-span of adrenalectomized rats (Rogoff and Stewart, 1928), (b) estrogens and androgens can be extracted from the adrenal cortex and are found in normal urine (Dorfman and van Wegeman, 1938). This may indicate that the hormones are derived, in part at least, from the adrenal cortex, (c) corticosterone and the sex hormones are closely related in chemical structure, (d) cortical extracts have been reported to cause precocious sexual maturity in rats (Corey and Britton, 1931), (e) the origin of the gonads and the cortex is from adjacent portions of the coelomic epithelium.

Thomas Addison first described Addison's disease in 1855. Addison ascribed this disease as a tuberculous lesion of the adrenals. Further experimental and clinical data have substantiated Addison's conclusions that the disease is due to adrenal insufficiency. However, tuberculous disease is found only in a portion of the cases. It has been found that the disease is due to the deficiency of the cortex rather than the medulla (Rogoff, 1932). The chief features, which closely resemble those seen in adrenalectomized animals are: (a) muscular weakness and langour, (b) low blood pressure and reduced circulation rate, (c) gastrointestinal disturbances, loss of appetite (anorexia), hypochlorhydria and vomiting, (d) pigmentation of the skin and mucous membranes, bronzing, tanning or a dirty brown

cutaneous discoloration being a classical symptom of the disease (Addison, 1855), (e) lowered metabolic rate, subnormal temperature, sodium loss and a rise in serum potassium, reduced blood volume, dehydration and loss of weight, (f) renal insufficiency with consequent rise in blood nonprotein nitrogen (Rogoff and Stewart, 1926), (g) depression of the sexual functions, and (h) hypoglycemia may occur and be the immediate cause of death. The discoloration is due to the excessive accumulation of the normal cutaneous pigment, melanin. The pigment granules are deposited in the basal granules of the epidermis. Ascorbic acid, which is present in relatively high concentrations in the adrenal cortex, inhibits pigmentation in plants. It remains to be shown, however, that a lack of ascorbic acid causes the bronzing in Addison's disease.

Unless patients with this disease are treated with cortical hormone or with a diet of high salt and low potassium, death occurs in one to three years. Unfortunately, the results of treatment are not good. In some cases the disease is not altered by treatment while in others remarkable improvement occurs.

MATERIALS AND METHODS

The Warburg manometer was used to measure the oxygen consumption of slices obtained from the adrenal gland. A detailed treatise of the Warburg procedure has been presented by Warburg (1926) and Dixon (1943).

The theory of the constant volume, differential pressure apparatus is as follows: all quantities of gas evolved by a reaction are recorded as positive pressures and all quantities absorbed as negative. The readings are correspondingly considered as positive or negative according to whether they indicate an evolution or an absorption of gas. In the manometer the reading is positive if the liquid rises in the open limb.

All gas volumes are expressed in cubic millimeters of dry gas at normal temperature and pressure. All pressure readings are in millimeters of the liquid in the manometer tube.

The Warburg equation may be derived as follows:

Let x = the amount of gas evolved in cubic millimeters at normal temperature and pressure. (If the gas is absorbed, x will be negative.)

h = the corresponding reading of the manometer.

V_g = the volume of the gas space in the vessel (which must include the connecting and manometer tubes down to the half-way point of the scale).

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h = the corresponding reading of the manometer

V_G = the volume of gas in the vessel (which must include the connecting manometer tubes down to the 150 mm. mark of the scale.

V_F = the volume of the liquid in the vessel.

T = the absolute temperature of the water-bath.

P = the initial pressure in the vessel (in general equal to the barometric pressure).

P_0 = the normal pressure (760 millimeters of mercury in millimeters of manometer fluid).

If D is the density of the fluid,

$$P_0 = 760 \frac{13.60}{D}$$

p = the vapor pressure of water at temperature T.

α = the solubility of the evolved gas in the liquid in the vessel (in cubic millimeters of gas [at normal temperature and pressure] dissolved in one cubic millimeter of liquid when in equilibrium with a partial pressure of the gas equal to P_0).

Then the initial amount of gas in the gas space = $V_G \frac{273}{T} \frac{P - p}{P_0}$, and the initial amount of dissolved gas = $V_F \alpha \frac{P - p}{P_0}$

Also the final amount of gas in the gas space = $V_G \frac{273}{T} \frac{P - p + h}{P_0}$

And the final amount of dissolved gas = $V_F \alpha \frac{P - p + h}{P_0}$

The total amount of gas finally present is the amount initially present plus the amount x produced. Therefore,

$$\left(V_G \frac{273}{T} + V_F \alpha\right) \frac{P - p + h}{P_0} = \left(V_G \frac{273}{T} + V_F \alpha\right) \frac{P - p}{P_0} + x,$$

Whence,

$$x = h \left[\frac{V_G \frac{273}{T} + V_F \alpha}{P_0} \right]$$

The expression in square brackets remains constant for a given gas with any given vessel, provided the liquid volume and the temperature remain unchanged. This quantity is known as the constant (K) of the apparatus. If the value of K is known and the reading h is taken, then by multiplication the amount of gas evolved in cubic millimeters of dry gas at standard temperature and pressure may be obtained. Thus no further correction of any kind is necessary.

$$x = hK$$

The above equation is equally valid if a second gas is present in the vessel in addition to the reacting gas.

The value of the constant (K) depends on the nature of the gas absorbed or evolved, and since the two gases involved in respiration, oxygen and carbon dioxide, have very different solubilities, the constant for oxygen may have an appreciably different value from that for carbon dioxide in the same apparatus.

Because one end of the manometer tube is open to the

air it is very sensitive to slight changes in the barometric pressure or the water bath temperature which may occur during an experiment. A change of a small fraction of a degree will alter the reading by several millimeters, and the barometer frequently changes fifteen or twenty millimeters of Brodie[1] per hour. It is, therefore, necessary to have not only a very efficient thermoregulator, but also an additional manometer to act as a thermobarometer. The latter is an extra Warburg manometer filled with water, the volume of which equals the volume of the reacting solutions in the other manometers. Whenever a reading is taken, the thermobarometer is also read and its reading subtracted algebraically from those of the other manometers in order to eliminate the errors due to changes in the external conditions. Since any change in the thermobarometer acts equally on all of the manometers, any changes will affect the readings of all the instruments in the same way. Therefore, with this correction, the readings are usually significant to the nearest one-half millimeter.

The thermoregulator was set so that the water bath was kept at a temperature of 37 degrees centigrade plus or minus 0.05 of one degree centigrade.

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[1] The composition of Brodie solution is as follows: 500 cubic centimeters of water, 23 grams of sodium chloride, 5 grams of sodium tauroglycocholate, and a few drops of an alcoholic thymol solution. The solution may be colored with methyl violet.

air it is very sensitive to slight changes in the barometric pressure or the water bath temperature which may occur during an experiment. A change of a small fraction of a degree will alter the reading by several millimeters, and the barometer frequently changes fifteen or twenty millimeters of Brodie[1] per hour. It is, therefore, necessary to have not only a very efficient thermoregulator, but also an additional manometer to act as a thermobarometer. The latter is an extra Warburg manometer filled with water, the volume of which equals the volume of the reacting solutions in the other manometers. Whenever a reading is taken, the thermobarometer is also read and its reading subtracted algebraically from those of the other manometers in order to eliminate the errors due to changes in the external conditions. Since any change in the thermobarometer acts equally on all of the manometers, any changes will affect the readings of all the instruments in the same way. Therefore, with this correction, the readings are usually significant to the nearest one-half millimeter.

The thermoregulator was set so that the water bath was kept at a temperature of 37 degrees centigrade plus or minus 0.05 of one degree centigrade.

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[1] The composition of Brodie solution is as follows: 500 cubic centimeters of water, 23 grams of sodium chloride, 5 grams of sodium taurocholate, and a few drops of an alcoholic thymol solution. The solution may be colored with methyl violet.

The adrenal glands used in the course of the experiments to be reported here were obtained through the courtesy of the Mobley Packing House of Albuquerque, New Mexico. The glands were removed from the animal approximately fifteen minutes after killing had been accomplished by a blow on the head. The adrenals were then placed in a buffer solution (usually Krebs buffer) and packed in ice until used in the laboratory.

In the laboratory the glands were cut into square pieces before slicing. The square pieces, containing both medulla and cortex, were then placed between ground glass plates and sliced with a razor blade to a thickness of about twenty microns with a wet weight of approximately 100 milligrams. To simulate freezing conditions the above procedure was carried out over ice.

The following solutions were prepared for use in the experiments to be reported in this paper.

Krebs Buffer, Solution I

Sterile distilled water = 100 cc.

$Na\ Cl$	= 0.702	GRAMS
$K\ Cl$	= 0.017	''
$Ca\ Cl_2 : 2H_2O$	= 0.025	''
$Mg\ Cl_2 \cdot 6H_2O$	= 0.016	''

Krebs Buffer, Solution II

Sterile distilled water = 100 cc.

$Na_2H\ PO_4 \cdot 12H_2O$	= 0.247	GRAMS
$Na\ H_2PO_4 \cdot H_2O$	= 0.043	''

The adrenal glands used in the experi- ments to be reported here were obtained through the cooperation of the Mobley Packing House of [illegible], [illegible]. The glands were removed from the animals within fifteen minutes after killing and [illegible] head. The adrenals were [illegible] in a [illegible] solution (usually Krebs buffer) and [illegible] to the laboratory.

In the laboratory the glands were cut into [illegible] pieces before slicing. The [illegible], [illegible] medulla and cortex, were then placed between ground glass plates and sliced with a [illegible] to a thickness of about twenty microns with a wet weight of approximately 100 milli- grams. To simulate [illegible] conditions the entire procedure was carried out over ice.

The following solutions were prepared for use in the experiments to be reported in this paper.

Krebs Buffer, Solution I

Sterile distilled water = 100 cc.

Na Cl	= [illegible] grams
K Cl	= [illegible]
$Ca Cl_2 \cdot 2H_2O$	= [illegible]
$Mg Cl_2 \cdot 6H_2O$	= 0.0[illegible]6

Krebs Buffer, Solution II

Sterile distilled water = 100 cc.

$Na_2H PO_4 \cdot 12H_2O$	= 0.[illegible] grams
$Na H_2PO_4 \cdot H_2O$	= 0.0[illegible]

An equal volume of Krebs buffer was pipetted into the thermobarometer.

The manometers, with their vessels, were fitted on the racks of the constant temperature bath with both limbs of the U-tube open to the atmosphere. The vessels were equilibrated for fifteen to thirty minutes in order to allow the temperature inside the vessel to reach the temperature of the bath.

In order to determine the rate of O_2 consumption, a sufficient number of thirty minute readings were taken before pouring the liquid from the side arm into the bottom of the vessel. This represents the non-stimulated respiration. After "dumping" enough readings were taken to determine the stimulated rate of O_2 consumption and the experiment was terminated.

Each reading in millimeters was then corrected for changes in barometric pressure as indicated by the thermobarometer and a running total of corrected pressures were kept. These running totals were converted by means of the Warburg equation to the gas evolved in cubic millimeters at standard temperature and pressure. The latter figures may be then plotted against time.

The tissues were removed from the Warburg vessel, the excess water was removed by blotting, and the slices were weighed on an analytical balance. This represents "wet weight." The dry weights were obtained by allowing the tissues to

remain in contact with the atmosphere for one week before weighing.

The amount of oxygen consumed in cubic millimeters per milligram of tissue per hour is known as the QO_2. The QO_2 is determined by dividing the amount of oxygen consumed in cubic millimeters by the wet or dry weight of the tissue. The graphs in this paper represent the QO_2 against time (Figures 2, 3, 4, 5, 6, and 7).

EXPERIMENTAL RESULTS AND CONCLUSIONS

Using the Boell-Needham Ultramicrorespirometer, Dr. D. G. Humm (unpublished) has found a stimulated oxygen consumption in adrenal gland slices upon the addition of tyrosine to a glucose-phenylthiourea mixture (Figure 1). Since this observation was of a preliminary nature, it was deemed advisable to extend this analysis to include: the distribution in various animals, some effects of pH on the system, the chemical specificity of the reaction using analogues of tyrosine and in general opening the field for further detailed investigation.

The results obtained from these determinations are presented in tables one through four. To facilitate consideration of the data, graphs have been drawn relating the amount of oxygen consumed in cubic millimeters per milligram of tissue to time (Figures 2, 3, 4, 5, 6 and 7). The results presented in figure one are those of Professor Humm.

In the original investigations carried out by Humm, a tyrosinase system was sought; glucose, phenylthiourea and tyrosine were used. He was looking for the following type of system.

[illegible]

Using the [illegible]

D. G. [illegible] a [illegible]

consumption in [illegible] upon the [illegible] of

tyrosine to a [illegible]

Since this [illegible], it was

deemed advisable to [illegible]

distribution to [illegible], some effects of

the system, the [illegible] specificity of the [illegible]

analogues of tyrosine and in general [illegible]

for further detailed investigations.

The results obtained from these [illegible] are

presented in tables and [illegible]. [illegible]

consideration of the data, [illegible] have been [illegible]

the amount of oxygen consumed. [illegible]

milligrams of tissue [illegible]

The results presented in figure one were [illegible]

Professor [illegible].

In the original [illegible]

a tyrosinase system was [illegible]

and tyrosine were used. [illegible]

type of system.

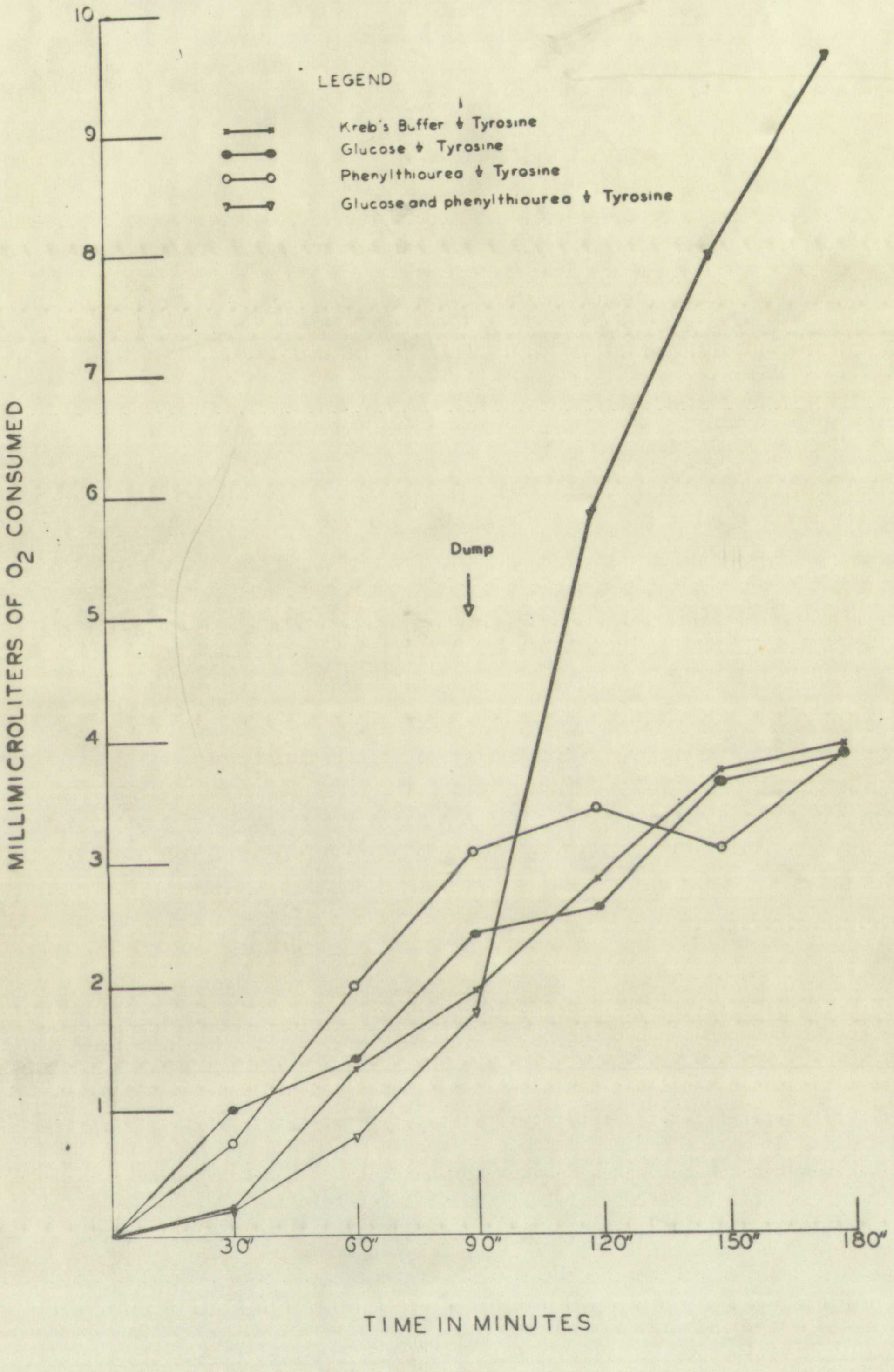
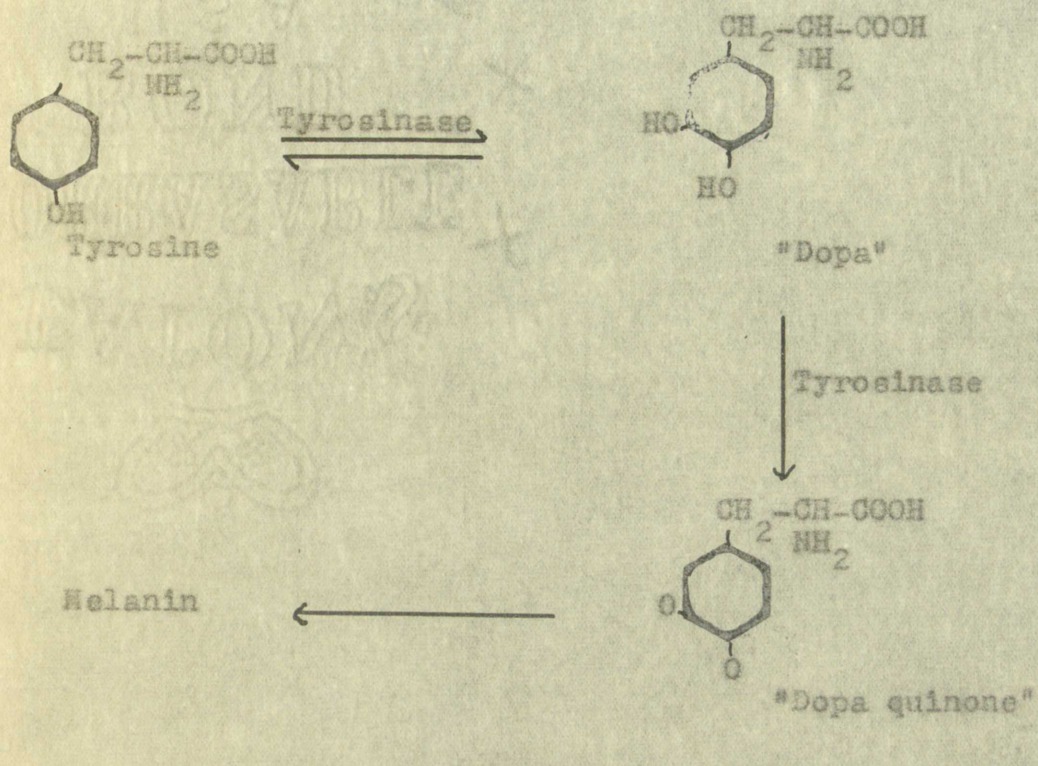


Figure 1. Oxygen Consumption - Rat Adrenal Slices.



From the mechanism that Humm postulated, one would not expect an increase in oxygen consumption upon the addition of tyrosine since phenylthiourea inhibits the metabolism of tyrosine to 3, 4 dihydroxyphenylalanine. However, the addition of tyrosine did cause an increase in respiration and this led to the speculation that tyrosine was being metabolized by some other path than that described by Nelson and Dawson (1946).

Using the constant volume differential pressure respirometer a marked increase in oxygen consumption of adrenal gland slices was noted upon the addition of tyrosine to a glucose-phenylthiourea mixture. However, upon the

addition of glucose to a phenylthiourea-tyrosine mixture the increase in oxygen consumption was not nearly as great. The same was true for the addition of phenylthiourea to a solution of glucose and tyrosine (Figure 2). Why the compounds had to be added in a particular order to get a marked increase in oxygen consumed was not understood. It may be the the result of an interaction between the tissue slice and the glucose and phenylthiourea. This interaction may either isolate or inhibit a system; thus making the oxidation of tyrosine possible and ultimately causing an increased oxygen consumption. The enhanced respiration may be due to the following type of system.

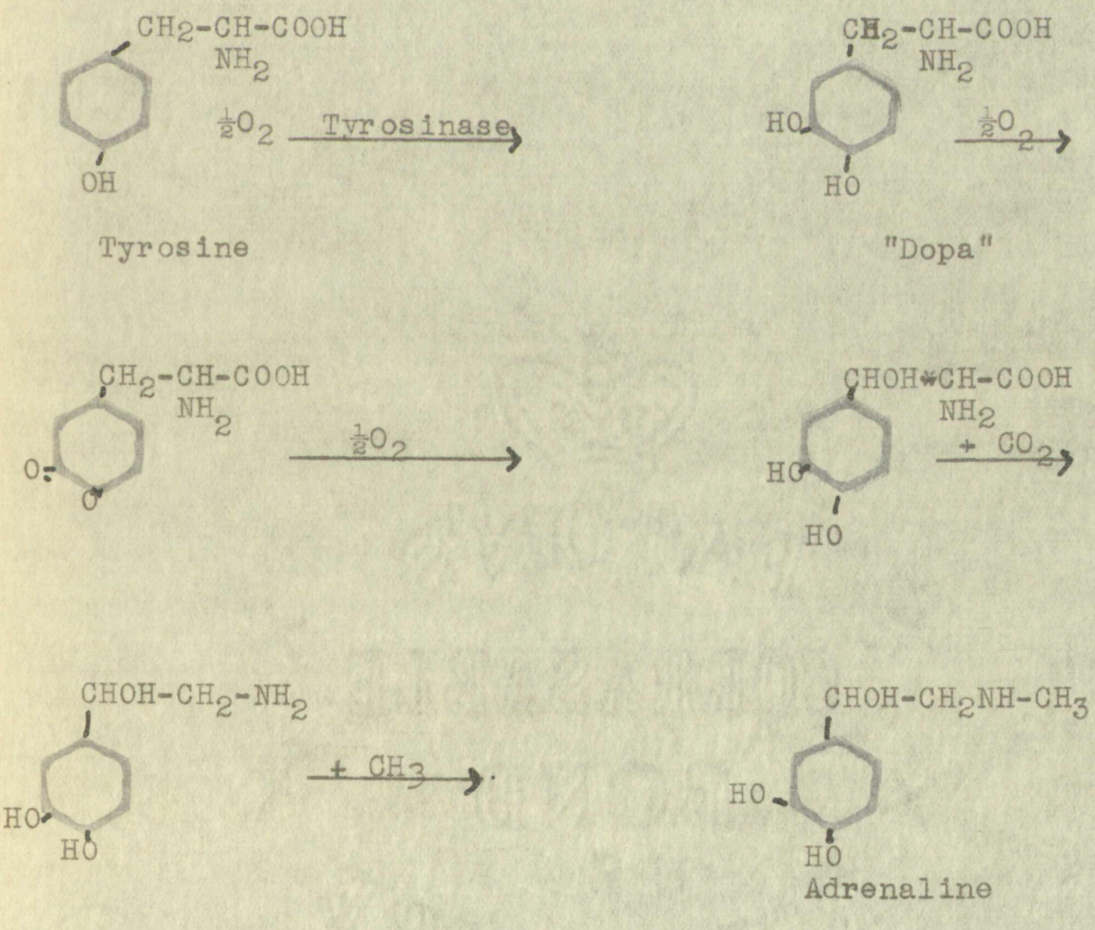


TABLE I

Q_{O_2} of Adrenal Gland Tissue from the Range Steer

No. of Animals	Average Tissue Wet Weight		
		1 c.c. of 1% Glucose and 1 c.c. of M/50 Phenylthiourea	
			1 c.c. of 120 mgm.% Tyrosine Added
7	49.8 mgm.	Q_{O_2} - Range 0.23-0.31 Average 0.27	Q_{O_2} - Range 3.2 - 4.8 Average 4.0
		1 c.c. of 120 mgm.% Tyrosine and 1 c.c. of M/50 Phenylthiourea	
			1 c.c. of 1 % Glucose Added
7	136.8 mgm.	Q_{O_2} - Range 0.06-0.15 Average 0.11	Q_{O_2} - Range 0.18-0.24 0.21
		1 c.c. of 120 mgm.% Tyrosine and 1 c.c. of 1% Glucose	
			1 c.c. of M/50 Phenylthiourea added
7	121.8 mgm.	Q_{O_2} - Range 0.24-0.52 Average 0.38	Q_{O_2} - Range 0.27-0.73 0.50

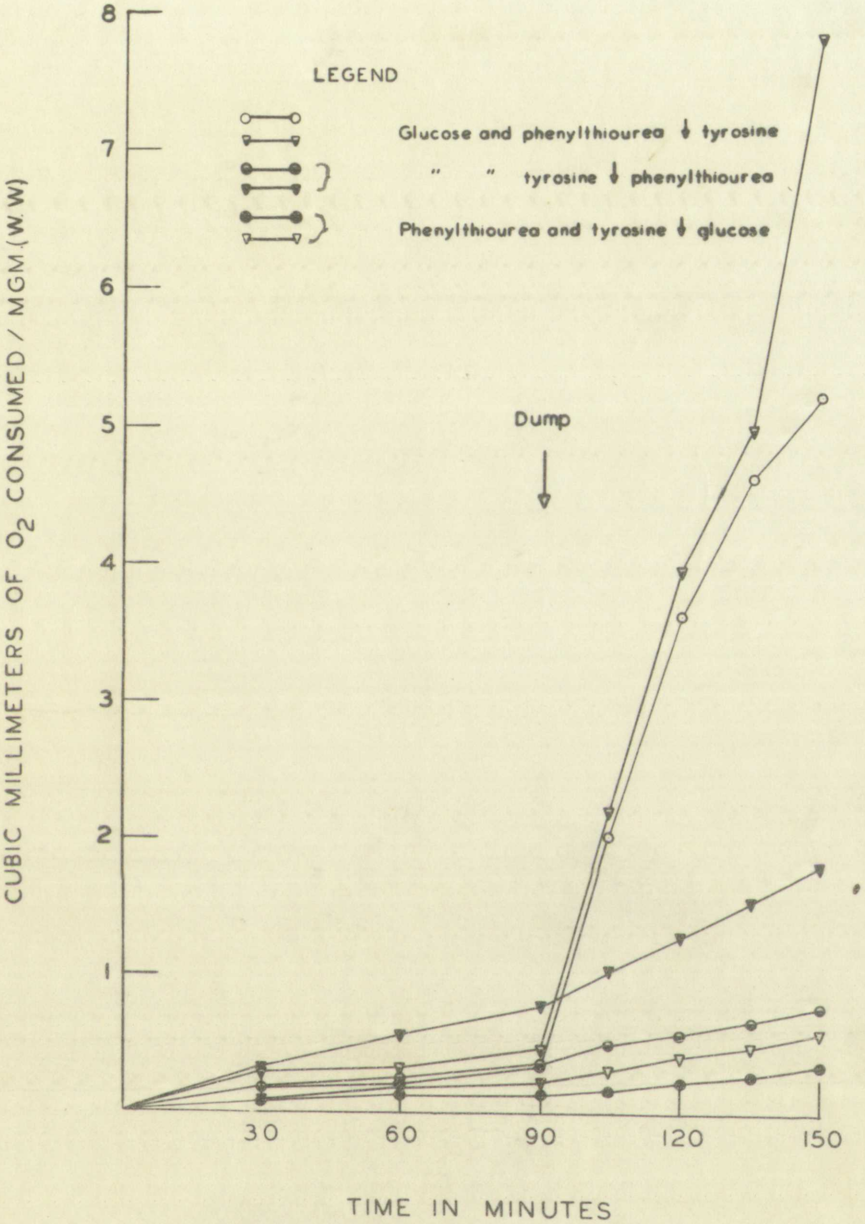
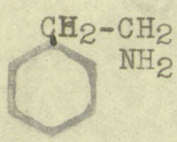
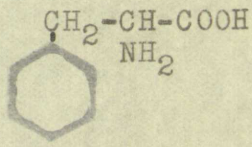


Figure 2. The Effect of Adding Solutions of Phenylthiourea, Glucose and Tyrosine in different Orders.

As can be seen by Figure (3), of the compounds tested thus far only tyrosine caused any stimulation. This fact was unexpected in that the structural formulae of phenylalanine and tyramine resemble that of tyrosine. The tyrosinase isolated from the potato or mushroom will react equally well with tyramine as well as tyrosine. The formulae of the above compounds are as follows:



Tyramine



Phenylalanine

However, the results of this experiment may not be valid in as much as the tyramine and phenylalanine were both dissolved in distilled water and the pH was not carefully controlled. In later results carried out by Humm, Watland and Roeder (unpublished), in which the pH of the reacting solutions was set at nine, a stimulation of the respiration of the adrenal slice was observed upon the addition of phenylalanine to a glucose-phenylthiourea solution. In these experiments the phenylalanine was dissolved in Krebs buffer.

As can be seen by Figure (3), of the compounds [illegible] thus far only tyrosine caused the [illegible]. This [illegible] was unexpected in that the structural [illegible] of phenylalanine and tyramine resemble that of tyrosine. [illegible] lated from the potato or mushroom will react [illegible] with tyramine as well as tyrosine. The formulas of the above compounds are as follows:

Phenylalanine   Tyramine

However, the results of this experiment [illegible] valid in as much as the tyramine and phenylalanine [illegible] dissolved in distilled water and the pH was not [illegible] controlled. In later experiments [illegible] and Hoeder (unpublished), in which the pH of the [illegible] solutions was set at pH [illegible], a stimulation of the [illegible] of the adrenal slice was observed upon the addition of phenylalanine to a glucose-phenylalanine [illegible]. In these experiments the phenylalanine was [illegible] in the buffer.

TABLE II

Q_{O_2} of Adrenal Gland Tissue from the Range Steer

No. of Animals	Average Tissue Dry Weight	1 c.c. of 1% Glucose and 1 c.c. of M/50 Phenylthiourea	
			1 c.c. of 120 mgm.% Tyrosine Added
7	13 mgm.	Q_{O_2} - Range 0.58-1.40 Average 0.99	Q_{O_2} - Range 19.70-25.10 Average 22.40
			1 c.c. of 120 mgm.% Tyramine Added
1	21.7 mgm.	Q_{O_2} - Range 0.9- 0.98 Average 0.94	Q_{O_2} - Range 0.90-0.99 Average .94
			1 c.c. of 120 mgm.% Phenylalanine Added
1	Average Tissue Wet Weight 85 mgm.	Q_{O_2} - Range 0.26-0.47 Average 0.36	Q_{O_2} - Range 0.35-0.44 Average 0.39

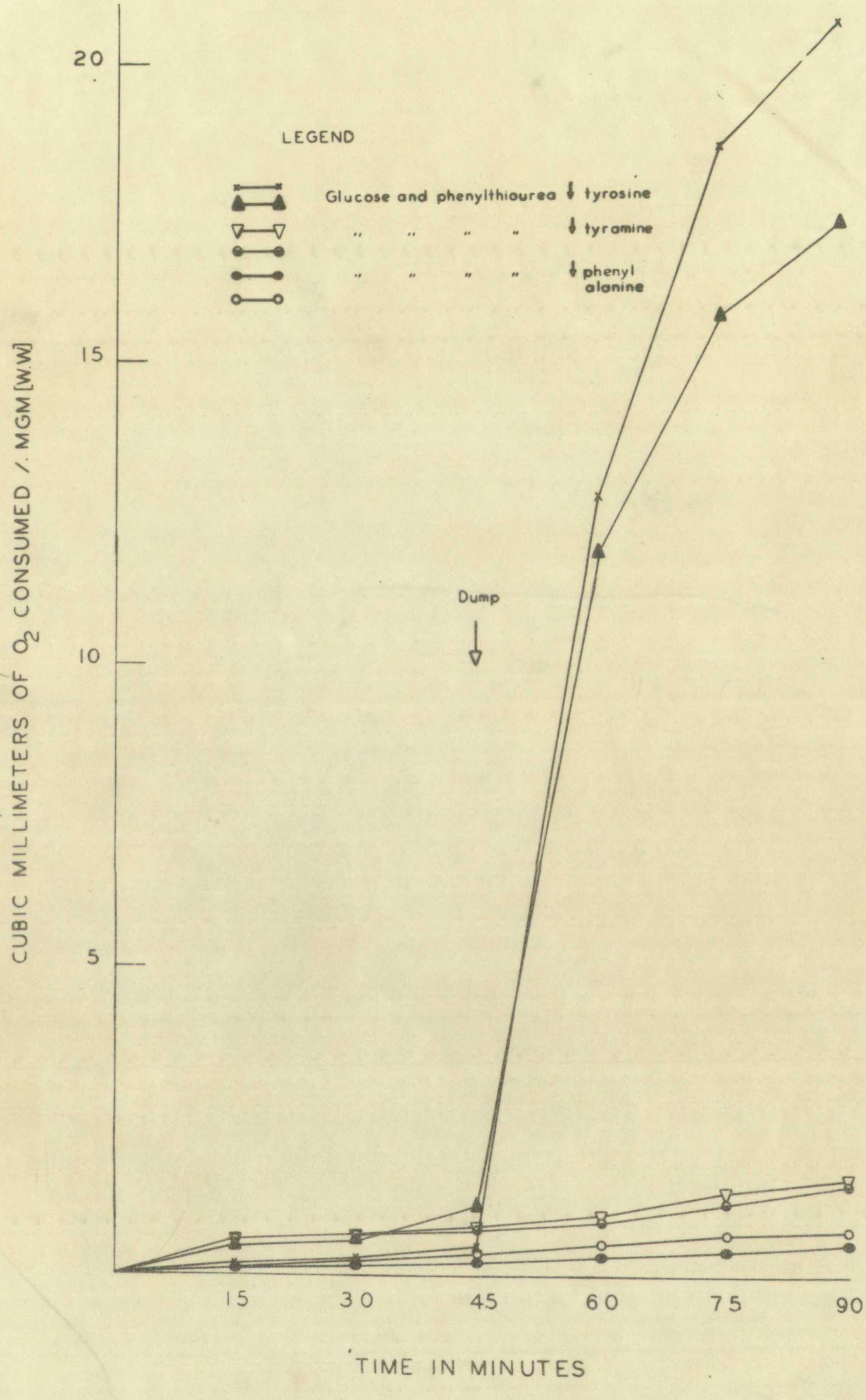


Figure 3. The Effect of Adding Chemical Analogues of Tyrosine.

It was not possible to check all of the chemical analogues of tyrosine due to their unavailability at this time. A later paper will deal with those compounds whose structural formulae resemble tyrosine.

One of the first steps in any investigation of an enzyme system is to prove the actual presence of an enzyme. Since these catalysts have been thus far all protein in nature, the true enzymatic activity should be destroyed upon boiling. Autoclaved wet tissues showed an appreciable decrease in respiration as compared to normally respiring tissue. (Figure 4). In most cases respiration is completely interrupted by boiling in water; however, in this instance the residual oxygen consumption may be due to a heat stable prosthetic group. It is well known that boiling will not destroy the metal ions (prosthetic groups) which are integrally associated with some enzymes (Warburg, 1950).

The effects of various poisons on this respiratory system will be covered in a later paper.

As can be seen in Figure 5 the amount of oxygen consumed increased as the solutions become more alkaline. However, the results from these early experiments may be open to question since the pH of all the solutions was not carefully controlled. The high final pH in some of the experiments can be accounted for by the fact that very basic

It was not possible to [illegible] the [illegible]
analogues of tyrosine [illegible] at this
time. A later paper will [illegible]
structural formulae [illegible].

One of the first steps in [illegible] of an
enzyme system is to prove [illegible]
Since these catalysts have been [illegible]
nature, the true enzymes [illegible]
boiling. Autoclaved [illegible] tissue [illegible] de-
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instance the residual oxygen [illegible]
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which are integrally [illegible]
1950).

The effects of [illegible]
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As can be seen in Figure [illegible] the amount of [illegible]
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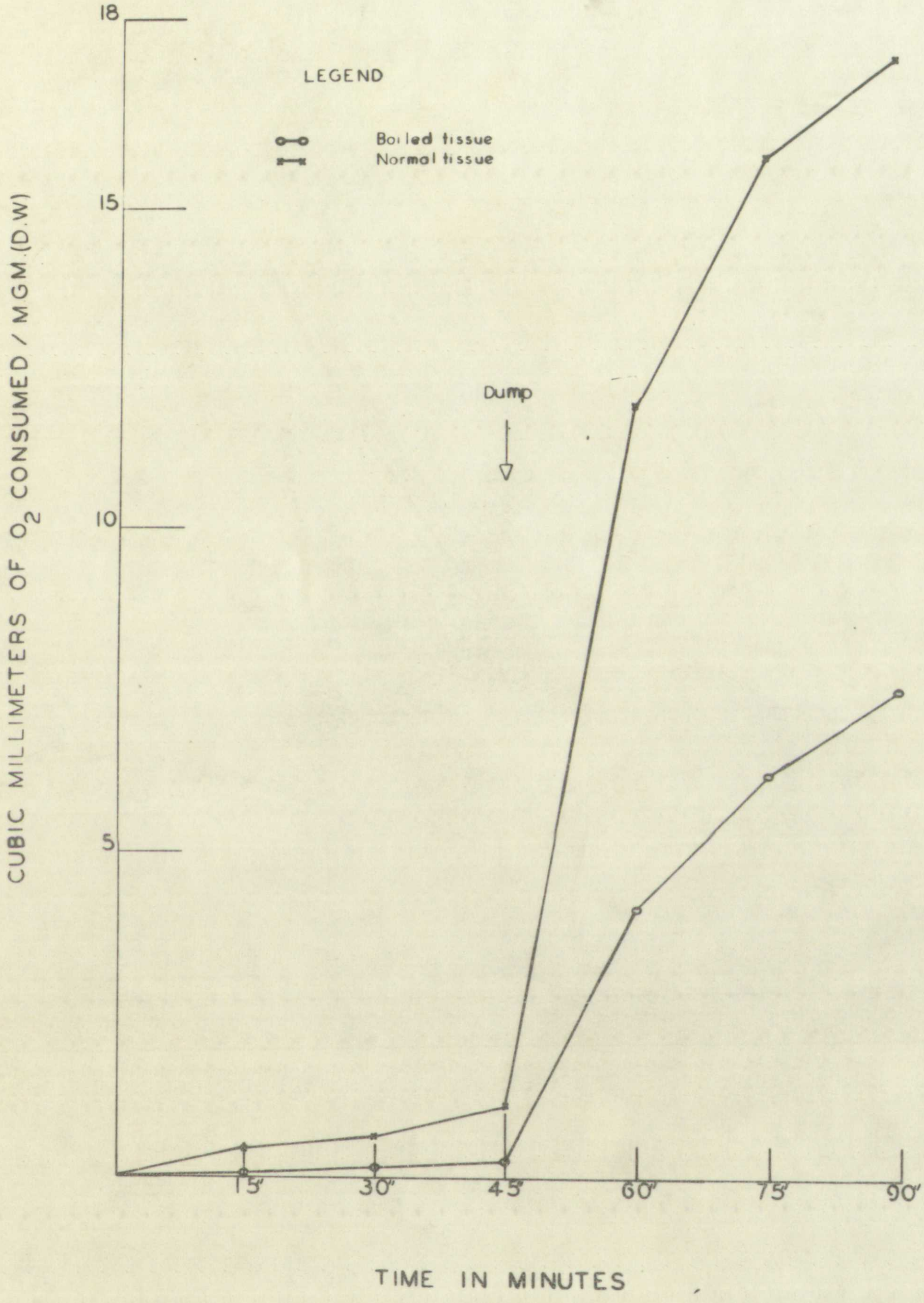


Figure 4. The Effect of Boiling on the Enzyme System in the Adrenal Gland. (Tyrosine added to a mixture of Phenylthiourea and Glucose.)

TABLE III

Effect of pH on the Q_{O_2} of Adrenal Gland Tissue from the Range Steer

No. of Animals	pH	Average Tissue Dry Weight	Before the Addition of 1 cc. of 120 mgm. % Tyrosine	After the Addition of 1 cc. of 120 mgm. % Tyrosine
1	6.7	4.80	1.70	13.80
1	9.5	5.10	3.05	32.10
1	10.9	12.00	0.63	26.80

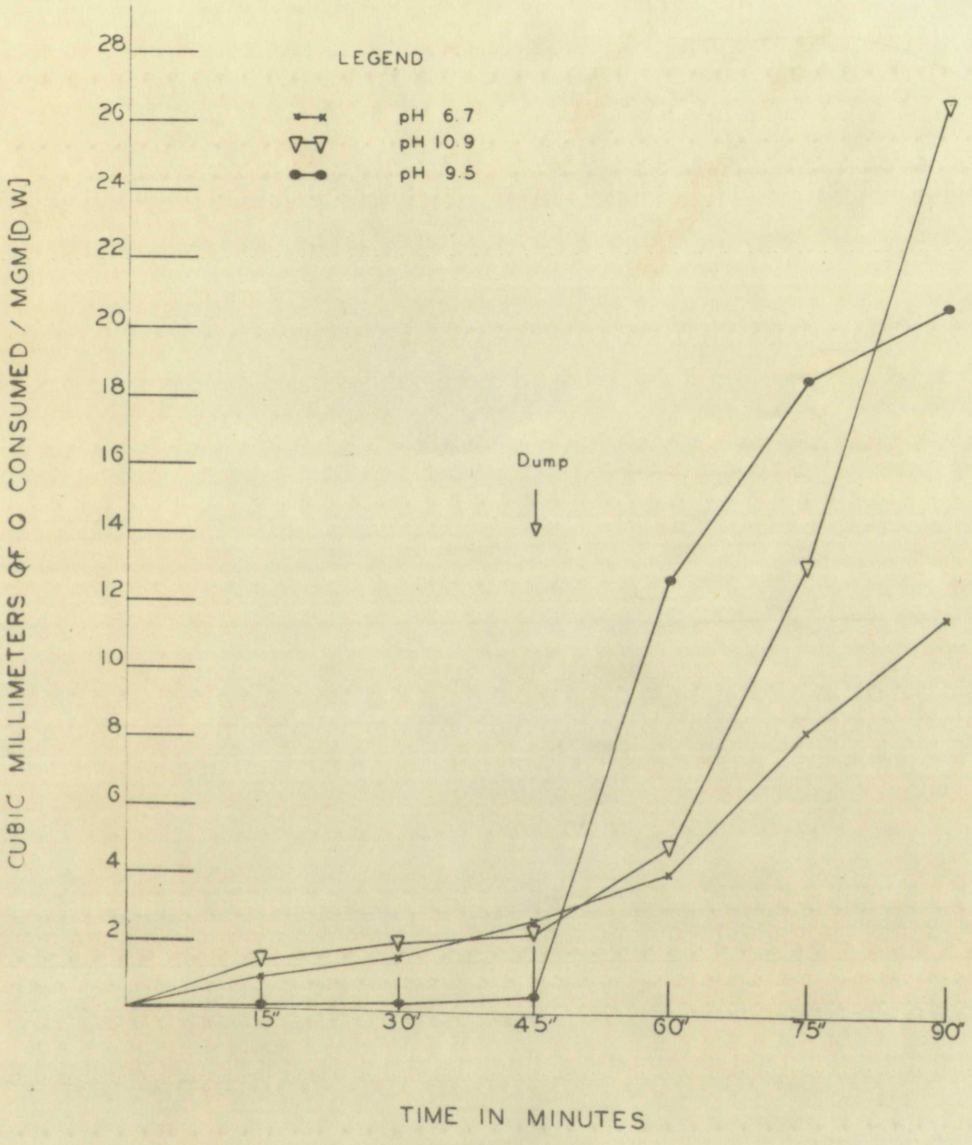


Figure 5. The Effect of pH on the Adrenal Gland Respiration. (Tyrosine added to a mixture of Phenylthiourea and Glucose.)

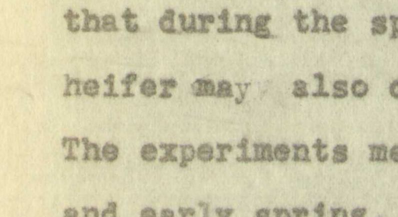
tyrosine was added to neutral glucose and phenylthiourea.

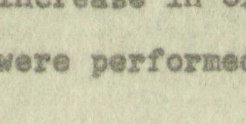
According to Watland and Roeder (unpublished) when all the solutions are adjusted to pH 9 an increase in oxygen consumption is noted and the final pH of the reacting mixture is less than nine.

It is interesting to note that a sex influenced factor is indicated (Figure 6). It can be seen that a mixture of tyrosine, phenylthiourea and glucose cause no noticeable increase in oxygen consumption when slices of the adrenal gland of the heifer are employed. The increased respiration in males may be due to the presence of some substance that is not found in the female. However, it must be remembered that seasonal variations in sexual activity may occur and that during the spring and summer the adrenal slices of the heifer may also cause an increase in oxygen consumption. The experiments mentioned were performed during the winter and early spring.

Sex influence leads to the interesting possibility that the sex hormones may in some way be responsible for the intensified respiration.

In as much as female sex hormones all contain a phenolic hydroxyl in the 3 position, and are substituted in the 1-6 positions, their formulae might possibly be written:





tyrosine was added to adrenal glands and [illegible].

According to [illegible] and [illegible] when all the solutions are adjusted to pH 7 an increase in oxygen consumption is noted and the pH of the reacting mixture is less than seven.

It is interesting to note that a similar effect in the is indicated (Figure 6). It can be seen that a mixture of tyrosine, phenylthiourea, and glucose causes no noticeable increase in oxygen consumption when slices of the adrenal gland of the heifer are employed. The increased respiration in males may be due to the presence of some substance that is not found in the female. However, it must be remembered that seasonal variations in sexual activity may occur and that during the spring and summer the adrenal glands of the heifer may also cause an increase in oxygen consumption. The experiments mentioned were performed during the winter and early spring.

Sex influences leads to the interesting possibility that the sex hormones may in some way be responsible for the intensified respiration.

In as much as female sex hormones all contain a phenolic hydroxyl in the 3 position, and are unsubstituted in the 1-6 positions, their formulas might possibly be written

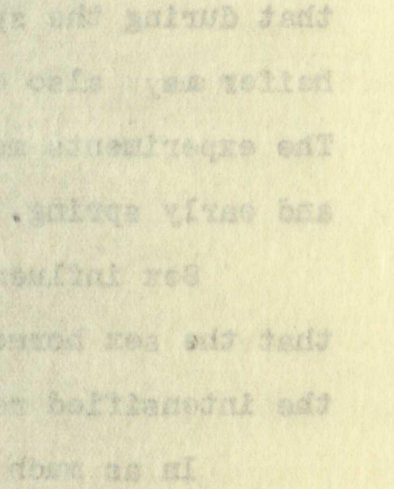


TABLE IV

Influence of Sex on the Q_{O_2} of Adrenal Gland Tissue in Hereford Cattle

No. of Animals	Average Tissue Dry Weight	1 c.c. of 1% Glucose and 1 c.c. of M/50 Phenylthiourea	1 c.c. of 120 mgm. % Tyrosine Added
Steer			
7	13 mgm.	Q_{O_2} - Range 0.58-1.50 Average 1.00	Q_{O_2} - Range 21.00-27.00 Average 24.00
Heifer			
4	20 mgm.	Q_{O_2} - Range 0.51-0.67 Average 0.60	Q_{O_2} - Range 1.10-1.20 Average 1.15

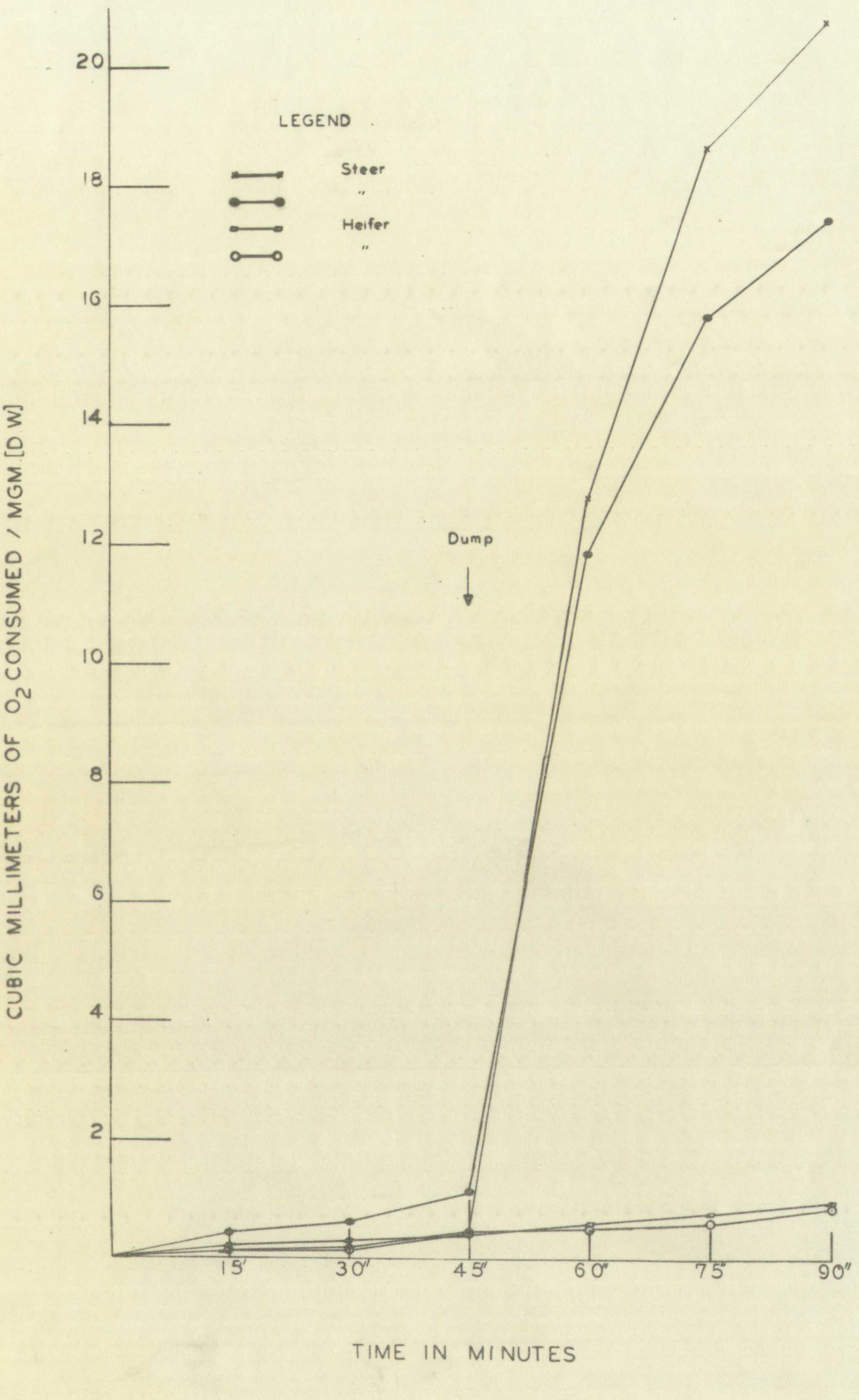
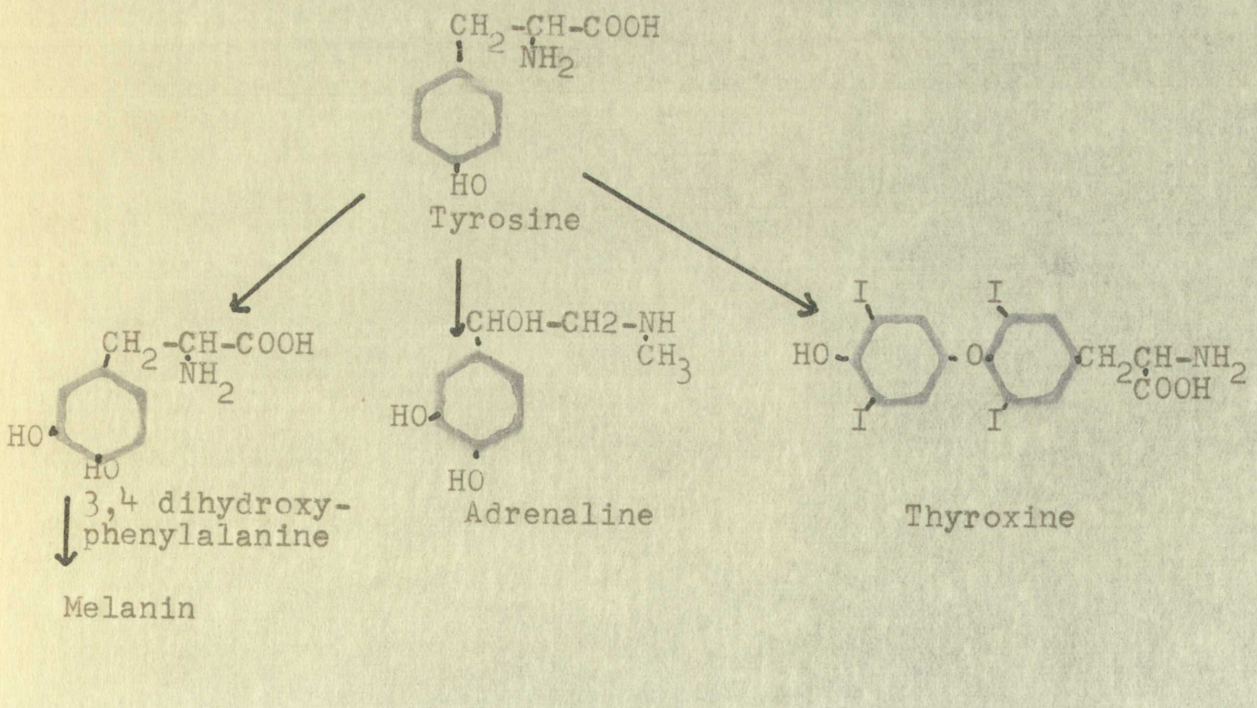
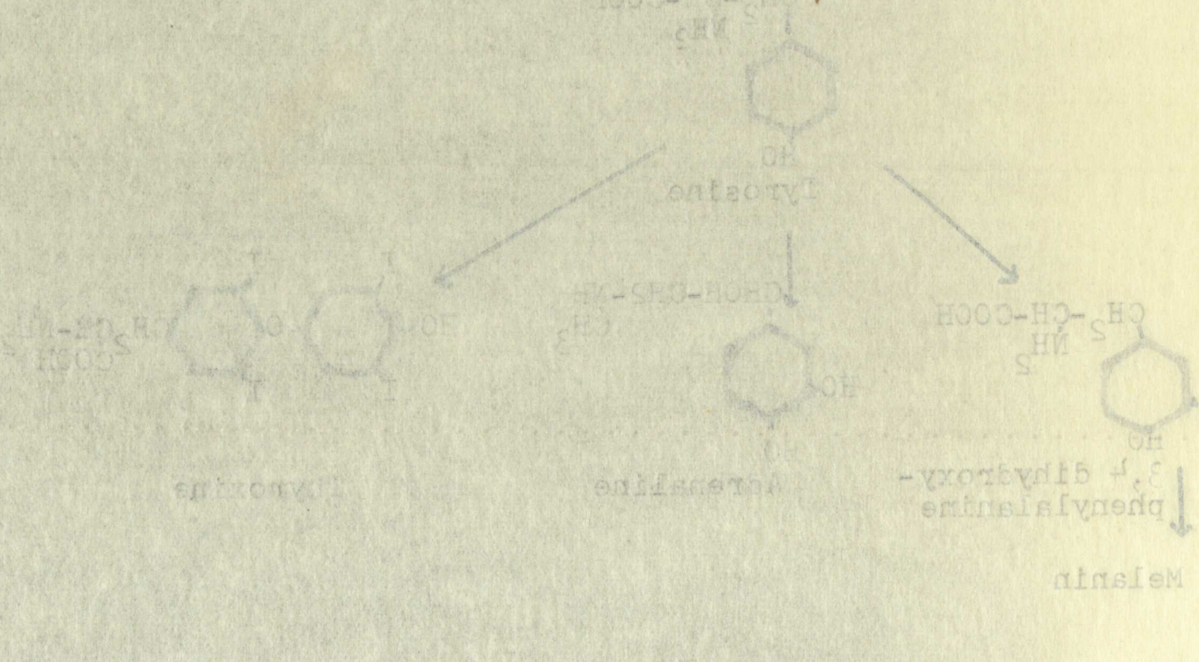


Figure 6. Oxygen Consumption of Adrenal Gland Slices-Steer and Heifer. (Tyrosine added to a mixture of Phenylthiourea and Glucose.)

One of the most fundamental problems in biology is the question of how particular structures develop their peculiar biochemistry. An embryological theory bearing on this question has been proposed by Professor D.G. Humm as part of an unpublished study concerning the neural crest. It is known that the neural crest, among its many derivatives, gives rise to the adrenal medulla and pigment cells in some animals. It has been shown (Nelson and Dawson, 1946) that tyrosine is the precursor of melanin in the potato and mushroom. Biochemical studies of the empirical formulae show that tyrosine resembles both adrenaline and thyroxine. Thus there is a possibility that tyrosine is acting as a precursor of adrenaline and thyroxine. The thyroid gland develops in an area where the cartilage forming activity of the neural crest is at a maximum; therefore it may be that the secretory cells of this gland are derived from the same presumptive area as the adrenal medulla and the pigment cells. Thus Dr. Humm has proposed the following scheme:



One of the most intriguing problems [illegible]
the question of how [illegible]
peculiar biochemistry. An [illegible] answer to
this question has been proposed by Dr. [illegible] Hume as
part of an unpublished [illegible] the neural crest.
It is known that the neural crest, among its many derivatives,
gives rise to the adrenal medulla and the pigment cells in
animals. It has been shown ([illegible]) that
tyrosine is the precursor of melanin in the pigment [illegible]
room. Biochemical studies of [illegible] show
that tyrosine resembles both adrenaline and thyroxine. Thus
there is a possibility that tyrosine is acting as a precur-
sor of adrenaline and thyroxine. The thyroid gland develops
in an area where the cartilage forming activity of the
neural crest is at a maximum; therefore, it may be that the
secretory cells of this gland are derived from the same
presumptive area as the adrenal medulla and the pigment cells.
Thus Dr. Hume has proposed the following scheme:



To return to the system under consideration in this paper, later results indicate that the adrenal medulla is the active principle since an increase in the oxygen consumed was observed only in the medulla upon the addition of tyrosine to a glucose-phenylthiourea solution (Humm, unpublished). If true, this observation would account for the fact that there was no noticeable increase in oxygen consumption when slices of the testis and kidney were used (Figure 7). The adrenal cortex, which does not show a detectable increase in respiration upon the addition of tyrosine, arises from the mesonephros which also gives rise to the testis and functional kidney.

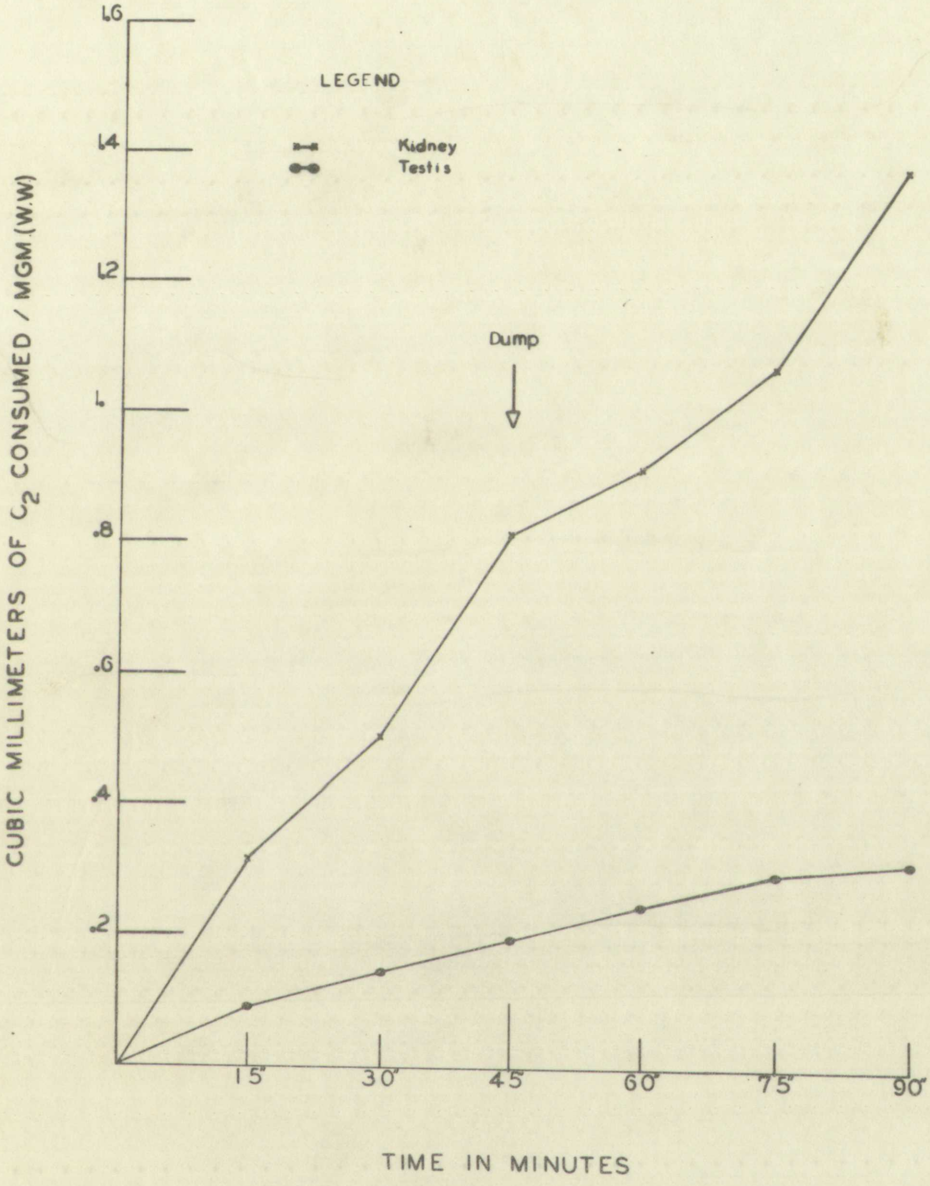


Figure 7. The Failure of Phenylthiourea, Glucose and Tyrosine to Stimulate Respiration in Kidney and Testis Slices.

SUMMARY AND CONCLUSIONS

Upon the addition of a solution of tyrosine to cattle adrenal gland slices in a solution of phenylthiourea and glucose, a stimulation of oxygen consumption was observed.

The increased oxygen consumption under these conditions was found to be dependent upon the pH of the solutions. As alkaline pH optimum was suggested.

Analogues of tyrosine, e.g., tyramine and phenylalanine, were tested and found to lack the capacity to stimulate the oxygen consumption of adrenal gland slices.

Based on observations made on the steer and heifer, a sex influence on the steer was suggested.

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