HOWARD JAMES DUNCAN

# HEPATIC CARBOHYDRATE METABOLISM IN THE DOMESTIC FOWL.

# GLUCOSE OXIDATION BY LIVER SLICES FROM THE DOMESTIC FOWL: ACTIVITY OF THE PHOSPHOGLUCONATE OXIDATIVE PATHWAY.

by

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# GENERAL INTRODUCTION

The onset of sexual maturity in the female domestic fowl is accompanied by increases of the amounts of various tissue and plasma constituents. There is much evidence that these changes are caused by secretion of estrogen from the ovary. Moreover, there is considerable evidence to support the view that these increases of tissue and plasma constituents represent material elaborated for transfer to the maturing ova in the ovarian follicles, i.e., material destined for the nutrition of the offspring.

A number of other tissues that carry out biochemical functions similar to those of avian liver, such as mammalian liver, mammary gland, and adipose tissue, have been shown to possess active phosphogluconate oxidative pathways. In these tissues, the activity of this pathway appears to increase in tandem with elevated synthetic activity, the pathway supposedly contributing to the tissue requirements for substrate and/or cofactor.

From a teleological standpoint, there would seem to be some degree of biological analogy between the functions of the liver and mammary glands of the mammal and those of the avian liver in relation to provision of nutrients for the offspring. If this analogy be

granted, then it would seem reasonable to expect an increased activity of the phosphogluconate oxidative pathway in the liver of the female domestic fowl with the onset of reproductive activity. Furthermore, the ability of estrogen to cause elevation of the levels of several tissue and plasma components may be related to any action estrogen might be shown to have on the activity of the phosphogluconate oxidative pathway.

The foregoing considerations prompted the studies of the phosphogluconate oxidative pathway in the liver of the domestic fowl that are the subject of the present thesis. At the same time, the absence from the literature of any previous reports of similar studies on an avian species was an additional reason for undertaking these studies.

Activity of the pathway was detected be measurement of the "in vitro" liberation of isotopically labeled carbon dioxide from specifically labeled glucose by liver slice technique.

# I. HISTORICAL REVIEW

## A. Lipogenesis in the Liver of the Domestic Fowl.

Avian liver, and in particular that of the domestic fowl, undergoes marked changes in gross composition when the bird comes into lay, the composition returning to the previous state when laying ceases. The onset of sexual maturity is also accompanied by the elevation of the levels of various plasma constituents.

The first indication that ovarian hormones are involved in the regulation of the blood fat in birds was obtained by Lawrence and Riddle (1916)(1), who noted that the blood fat is markedly increased when hens are in lay. This observation was soon confirmed by Warner and Edmond (1917)(2), who showed that the degree of elevation of blood fat of hens, at the onset of laying and during the laying period, was even greater than that first reported by Lawrence and Riddle.

This basic observation of striking increases in blood lipid levels during periods of ovarian activity, has been often confirmed and much elaborated upon by many workers, including Heller et al.(1934)(3), Greenberg et al.(1936)(4), Lorenz et al.(1938)(5), Chaikoff et al. (1941)(6), Riddle (1942)(7), and Walker et al.(1951)(8).

The first evidence that both estrogen and gonadotrophin increase the plasma fat of the fowl was put forward in 1938, in a series of papers by investigators at the University of California (9). Estrogen, which the authors had obtained from pregnant mare serum, was shown to be capable of practically doubling the plasma lipids of pullets or cockerels, 12 to 24 hours after administration. All of the lipid constituents of the blood shared in the increase. It was also discovered that the blood lipids could be greatly increased by prolonged stimulation of the ovaries of pullets with gonadotrophic hormone. The work of these authors, in addition, led to the substantiation of the very high levels attained by the plasma lipids of fowl in the active laying state. Furthermore, it was discovered that there was an accompanying increase in liver fat during this period of ovarian activity.

In studies concurrent with those cited above, Lorenz et al.(1938)(5) produced lipemia in immature birds of both sexes by administration of crude estrin. The lipemia was detectable within 12 hours of the estrin treatment. Zondek and Marx (1939)(10) reported extreme elevation of the blood lipids of cocks following treatment with large doses of the estrogens, estradiol and diethylstilbestrol. The authors found, in these experiments,

that the total liver lipid of the birds was also increased. In 1940, Entenman et al.(11), who injected estradiol benzoate into immature male and female chickens, likewise found large increases in all of the lipid fractions, the values approaching or exceeding those they reported in laying females. Similar results have been reported for cocks, capons, pigeons, and ducks (Riddle 1942 (12); Benoit 1950 (13)). These effects of estrogen have been confirmed for the domestic fowl by Landauer et al.(1939)(14), Flock and Bollman (1942)(15), Common et al.(1947)(16), and Lorenz and Bachman (1946 (17), 1947 (18)), using a variety of estrogenic compounds.

A question that arose from the above work concerned the origin of the lipid material that appeared in the blood and liver of the fowl, at the onset of sexual maturity or following the administration of estrogen or gonadotrophin.

That the lipemia does not depend on dietary fat has been demonstrated by the production of estrogen-induced lipemia after withholding food from birds for 48 hours (Zondek and Marx, 1939)(19). Bird's work (1946)(20) helped to rule out exogenous fat as the source of the lipid. It was found that estrogen does not promote more efficient absorption of fat from the gut of the hen. These conclusions were confirmed by Baum and Meyer (1956)(21),

who demonstrated a lipemic response in hens on a fat-free diet. These observations eliminated the possibility of dietary fat being responsible for the lipemia, and suggested rather the involvement of a metabolic synthetic pathway.

Chaikoff (1941)(6), studying the domestic fowl in puberty, showed that fat deposition in the liver may precede the development of lipemia. This work gave rise to the suggestion that the blood lipids were derived from lipids synthesized in the liver. Hevesy and Hahn (1938)(22) had reached the same conclusion from a consideration of the relative lipid phosphorus specific activities of liver and plasma of hens injected with $P^{32}$.

Taurog (23), in 1944, discovered that there was a significant increase in the "in vitro" formation of phospholipid by the hen liver after the birds had been treated with stilbestrol. This observation suggested that estrogen causes, through a direct or indirect mechanism, increased lipogenesis by the liver and ultimately makes the lipemia possible.

That the liver is the principal organ concerned with the increased synthesis of lipids, induced by estrogen, was revealed by Ranney and Chaikoff (1951) (24)(25) who demonstrated that estrogen does not cause lipemia in the functionally hepatectomized fowl.

A decided decrease in the concentration of blood phospholipids and total fatty acids was observed six hours after excluding the liver from the circulatory system of estrogen-treated birds. In sham-operated birds the values for phospholipid and total fatty acid increased during this period. This work was later confirmed and extended by Vanstone et al.(1957)(26).

Recent work by Hawkins and Heald (1966)(27) has clearly shown that the liver of the laying hen is capable of synthesizing greater quantities of triglyceride from fatty acids than is the liver of the immature bird. These authors have calculated that, in the laying bird, the overall rate of neutral lipid synthesis in the liver is some fifteen times that in the liver of the immature bird, and at the point of lay it can attain a level twenty-five times that of the immature fowl. The importance of the liver in the synthesis of triglycerides, therefore, would appear to be solidly established.

It is important to keep in mind the distinction between fatty acid synthesis and the synthesis of phospholipids or triglycerides, when making reference to lipogenesis or lipid synthesis. The evidence indicates that the liver plays an important role in the elevation of the rate of triglyceride and phospholipid synthesis, but its contribution to the increased levels of fatty acids, at the point of lay or after estrogen administration, has not been ~~equally~~

clarified to the same extent.

The liver of the domestic fowl has been shown to possess the enzymes of the non-mitochondrial system for the synthesis of fatty acids. The enzyme system was first isolated from pigeon liver by Gurin (1952)(28) and his collaborators, and was described by Wakil (1957)(29) in a series of papers that originated from the Enzyme Institute at the University of Wisconsin. The same system, or pathway, has been prepared from chicken liver by Tietz (1957)(30).

Ganguly (1960)(31), in a study of a wide range of tissues, found pigeon and hen liver to be the most active sources of the non-mitochondrial fatty acid synthesis pathway. This author also points out the lack of activity of the ovaries and of the oviduct of the laying hen, in contrast to the high activity of this enzyme system in the bovine mammary gland. This would imply that whereas some of the fat secreted in milk is synthesized by the mammary gland, the fatty acids in egg yolk are synthesized outside the ovaries or oviduct and most probably, in Ganguly's opinion, in the liver.

Infante et al.(1963)(32) found that liver from estrogenized cockerels incorporated more $^{14}$C-acetate into the fatty acids of phospholipids and triglycerides than did the liver from untreated cockerels. Experiments of this type, however, though indicative of greater fatty

acid synthesis after estrogen treatment, do not demonstrate that the actual synthetic process is enhanced. The possibility always exists that the permeability of the tissue to the added isotopically labeled precursor is greater with tissues derived from estrogen-treated or laying birds.

The general view at the present time would seem to be that the depot fats play a major role in supplying fatty acids for conversion into phospholipid and triglyceride by the liver (Heald)(33). This does not, however, exclude the possibility that the liver increases its rate of fatty acid synthesis when the hen comes into lay, in anticipation of the requirement for egg yolk materials. The observations of Ranney and Chaikoff (24,25) of the free fatty acid levels in sham-operated and functionally hepatectomized birds, as well as Infante's study (32) of "in vitro" incorporation of $^{14}C$-acetate into the fatty acids of phosplolipid and triglyceride by liver slices from estrogenized cockerels, support this latter view.

## B. Cholestrol Synthesis in the Liver of the Domestic Fowl.

Warner and Edmond (1917)(34) were the first investigators to present evidence that revealed higher plasma cholesterol levels in laying than in non-laying hens.

In 1933, Kaishie (35) reported that there was no difference between the cholesterol contents of the blood of non-laying female and of male birds. A comprehensive study of the influence of age, sex, and ovarian activity on the blood lipids of the domestic fowl,was later carried out by Lorenz et al.(1938)(9). They reported that with the onset of sexual maturity, the phospholipids and other fats of the blood increased sharply, whereas the cholesterol level increased only moderately.

Fleischmann and Fried (1945)(36) have asserted that estrogen does not increase the amount of cholesterol in the tissues, and that the increase observed in the plasma is due not to changes in synthesis or to destruction of cholesterol, but to alteration in the distribution of cholesterol between plasma and tissues.

Evidence contradictory to Fleischmann's view was presented by Stamler et al.(1950)(37) who found that diethyl-

stilbestrol pellets implanted into immature cockerels increased the cholesterol and fatty acids not only in the plasma, but also in the tissues. These authors studied the liver, kidneys, heart, adrenals, gut, and lung, all of which showed an increased cholesterol content. It was concluded from this work that estrogen increases cholesterol synthesis in the liver. Furthermore, Chaikoff et al.(38) had reported in 1948 that all lipid fractions, including cholesterol, share in the estrogen-lipemia response.

In 1951, Forbes and Petterson (39) also presented data that showed an estrogen-induced increase in cholesterol, as did Stamler et al.(1955)(40), although estrogen, again, was shown to increase the other lipid fractions more than it increased the cholesterol level. The observation that cholesterol shares in the estrogen lipemia response was later confirmed by Baum and Meyer (1956)(21).

It is now considered that cholesterol levels do not appear to be influenced appreciably by endogenous estrogen, since the differences between adult males, and laying, and non-laying females are not consistent, but that estrogen administration does lead to an increased level of cholesterol in the blood and tissues (Sturkie 1965)(41).

C. Protein Synthesis in the Liver
of the Domestic Fowl.

Research over a number of years has revealed that the level of plasma protein, as well as the level of plasma lipid, depends on the physiological condition of the fowl. Rochlina (42), in 1934, reported that higher plasma protein levels exist in mature hens than in cocks or chicks. The work of Sturkie and Newman (1951)(43) and Brandt et al. (1951)(44), along with that of Rochlina, has led to the acceptance that the level of the plasma proteins is lower in adult cocks than in the hen. This difference also exists between the immature cock and the pullet (Perk et al. 1960)(45).

Sturkie and Newman (1951)(43), however, were unable to detect any significant difference between the total plasma protein levels of groups of laying and non-laying White Leghorns, but they did note considerable variation among individuals in the different groups. The plasma protein levels of some laying hens were found to be close to the level observed in some birds that had been non-laying for two months.

A peak in the amount of protein in the plasma of pullets beginning to lay for the first time was observed

by Greenberg et al.(1936)(46). In 1955, Vanstone et al. (47) also reported a peak in the amount of protein in the plasma about the time that the fowl came into lay, the total plasma protein levels of the laying birds subsequently falling to levels not greatly different from those of cockerels. Bell and McIndole (1962)(48) later reported a protein peak in the plasma of two-year old hens during the ten day period before laying commenced. This increase in plasma protein has been shown to be due to the presence of phosphoprotein (Bell and McIndole 1962)(48) (Heald and Badman 1963)(49). Finally, Reznichenko (1962)(50) has reported that in hens laying down egg albumen, the plasma protein level was higher than at other times.

In addition to causing an increase in blood lipids, estrogen has been shown to induce elevation of the plasma protein level (Sturkie 1951)(51). Hosoda et al.(1954)(52) (1955)(53) later reported an estrogen-stimulated increase in the serum vitellin levels of maturing pullets. This study was later confirmed by Vanstone et al.(1957)(26).

The liver hypertrophy of estrogen-treated birds is associated not only with lipid deposition, but there is also a considerable increase in total liver protein (Common et al. 1948)(54). It became of interest to ascertain whether this increase of liver protein represented cellular hyperplasia or cellular hypertrophy. Accordingly,

Chapman et al.(1949)(55) investigated the changes in liver RNA and DNA consequent upon treatment of sexually immature pullets with estrogen. The results indicated considerable increases of the ratio RNA/DNA and of the total liver RNA, and a possible slight increase of liver DNA. The results, therefore, supported the view that cellular hypertrophy was the major effect and that estrogen stimulates the liver cells to synthesize protein more intensively. In 1951 Common et al.(56) followed up this work with a series of confirmatory studies including observations on a group of normal male and female birds carried from hatching to sexual maturity. This evidence for increased protein synthesizing activity was admitedly indirect, but it was nevertheless consistent and in conformity with the established behaviour of tissue cells known to be actively engaged in protein synthesis.

The key role that the liver plays in the response to estrogen was made evident by the work of Vanstone et al. (1957)(57), who found that functional hepatectomy abolishes the increase in plasma protein and phosphoprotein, as well as the incorporation of inorganic phosphate into phosphoprotein. This experiment demonstrated that all of the phosphoprotein is formed in the liver of the chicken.

The evidence therefore indicates increased protein synthesis by the liver, immediately prior to, and in conjunction with, the commencement of egg laying.

## SUMMARY

The foregoing review amply points out the important role of the liver in the biochemical events that culminate in egg-laying. With the onset of sexual maturity, the liver synthesis of all of the lipid fractions, and of some protein fractions, is enhanced. This enhancement of synthetic processes requires that certain substrates and cofactors be available in increased amounts for utilization by the enzyme systems involved.

One important cofactor that is expected to be required in increased amount as lipid and protein synthesis increases, is reduced NADP. NADPH has been demonstrated to be actively generated by the phosphogluconate oxidative pathway in tissues or organisms that possess this oxidative route and require this cofactor.

Circumstantial evidence suggests that this alternate glucose oxidation pathway may play an important role in supplying NADPH when the domestic fowl becomes capable of egg-laying.

## D. The Phosphogluconate Oxidative Pathway.

The reversible Embden-Meyerhof glycolytic pathway and the tricarboxylic acid cycle were long considered to be the only metabolic routes of glucose degradation. The work of Embden, Meyerhof, Parnas, and Warburg, led to the elucidation of the steps of the glycolytic scheme (58)(59), while the more important contributors to the formulation of the aerobic tricarboxylic acid cycle were Krebs and Johnson (60).

Suggestions of additional or alternate pathways of glucose oxidation, arose from a number of observations. Reagents such as iodoacetate, arsenite, or fluoride, which block component reactions in the glycolytic pathway, did not always inhibit glucose utilization completely; in some systems the inhibition was relatively slight (61-64). There was also the discovery in the early ninteen thirties of the Zwischenferment, glucose-6-phosphate dehydrogenase, and of 6-phosphogluconate dehydrogenase (65,66,67). Furthermore, carbohydrates other than those identified as intermediates of the two established pathways, and their derivaties, were being discovered, and it was not clear how they might be related to the classic glycolytic route (68).

The existence of the phosphogluconate oxidative pathway was established initially by the research of Warburg, Dickens, Lipmann, and Dische. Its elucidation followed largely from the efforts of Cohen, Horecker, and Racker (68,69,70,71,61). The recognition of this alternate glucose oxidation pathway was followed by considerable work on its distribution in animal tissues, plants, and microorganisms. The search revealed that a great number of organisms and tissues possessed the enzymes and the intermediates of this pathway (68,69).

The next step, although there is overlapping in time in the phases here described, was the attempt to evaluate the role of the phosphogluconate oxidative pathway, as compared with that of the Embden-Meyerhof route and the tricarboxylic acid cycle. The attempts were based upon the metabolism of glucose and other substrates labeled with $^{14}C$. Numerous efforts were made to develop experimental designs which would permit quantitative measurement of the contribution by the alternate oxidative route to the overall glucose oxidative process (68,69,72). One approach has been to determine the $^{14}CO_2$ production when glucose-1-$^{14}C$ and glucose-6-$^{14}C$ are metabolized by tissues (73). Bloom and Stetten, the originators of this technique, found that the yields of $^{14}CO_2$ from glucose-1-$^{14}C$ and

glucose-6-$^{14}C$ were about equal when incubated with kidney slices. Liver slices from the rat, however, yielded more $^{14}CO_2$ from glucose-1-$^{14}C$ than from glucose-6-$^{14}C$. A similar result was obtained by Muntz and Murphy with the liver of rats "in vivo"(74).

This often-utilized method of Bloom and Stetten was used in the work described in this thesis. The underlying theory is that $^{14}C$-1 and $^{14}C$-6 of glucose both form the methyl group of pyruvic acid in the glycolytic pathway, and, as a result, give the same amount of $^{14}CO_2$ when the pyruvic acid is oxidized by the Krebs tricarboxylic acid cycle. In the oxidation of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by the phosphogluconate oxidative pathway, however, C-1 is evolved as $^{14}CO_2$ in the first cycle and the C-6 becomes C-3 of glyceraldehyde phosphate. The glyceraldehyde phosphate may enter the glycolytic pathway and undergo various metabolic alterations, or be recycled through the alternate oxidative pathway. If the phosphogluconate oxidative pathway is present and operating in a tissue, glucose-1-$^{14}C$ would be expected to yield more $^{14}CO_2$ than glucose-6-$^{14}C$.

The pitfalls inherent in such quantitative measurements of the phosphogluconate oxidative pathway have been described by Wood (69,75), Racker (76) and Horecker (70), and require that the semiquantitative nature of the measurement be taken

into account in the evaluation of results. The method of Bloom and Stetten, however, is accepted as providing sound qualitative evidence for the existence of the pathway. In general, results which indicate exclusive participation of the glycolytic and tricarboxylic acid pathways are regarded as good evidence for the absence of a functional phosphogluconate oxidative pathway (i.e. ratio=1.0)(68).

The absence of a sound quantitative method for assaying the contribution to overall glucose oxidation by the phosphogluconate oxidative pathway has meant that the attribution of significance to this alternate route has also come to depend on the importance that accrues to the pathway by virtue of its products. It is believed that one of the most important functions of this alternate route is to provide NADPH, which in turn serves as a reducing agent in the synthesis of fatty acids, steroids, and various other substances (77). This theory is consonant with the distribution of the enzymes concerned with these synthetic processes. Liver (mammalian), mammary gland, testis, and adrenal cortex, are active sites of fatty acid and/or steroid synthesis, whereas these processes are not prominent in the metabolism of striated muscle, a tissue in which the existence of the phosphogluconate oxidative pathway has not been established unequivocally (78,79,80).

McLean (81) has shown that the formation of $^{14}CO_2$ from glucose-1-$^{14}C$ in mammary gland slices is increased during the period of lactation. The elevated activity of the alternate oxidative route coincides with elevated synthesis of fatty acids. The effects of physiological conditions on the ratio of $^{14}CO_2$ from glucose-1-$^{14}C$ : $^{14}CO_2$ from glucose-6-$^{14}C$, in rat liver slices, has been studied by Agranoff (82). It was found that with slices of maternal liver obtained during pregnancy, the contribution of the phosphogluconate oxidative pathway was increased. Adipose tissue affords another example of an apparent relationship between lipogenic activity and glucose metabolism which is in accordance with the hypothesis that the NADPH from the phosphogluconate oxidative pathway is utilized for fatty acid synthesis (83). Indeed, this relationship is even more striking in adipose tissue than it is in liver tissue.

Adrenal cortical tissue, which has the specialized function of synthesizing steroids, contains a relatively high concentration of glucose-6-phosphate dehydrogenase (84). The liberation of $^{14}CO_2$ from specifically labeled glucose has verified the presence of an active phosphogluconate oxidative pathway in this tissue also. Work by Koritz (85) supports the idea that enhancement of the alternate oxidative pathway in the adrenal is associated with increased

synthesis of the adrenocortical hormones.

The reduced NADP produced by the phosphogluconate oxidative pathway may also be important for reasons other than its usefulness as a requirement for fatty acid and steroid synthesis. NADPH is specifically required in reductive carboxylation of pyruvic acid to malic acid, the important reaction by which regeneration of 4-carbon dicarboxylic acids of the tricarboxylic acid cycle may be carried out in tissue that is synthesizing protein (86).

Another important factor is that the pathway represents a mechanism for the formation of pentoses, possibly for nucleotide synthesis. The pathway has been detected in Escherichia coli, where it is believed to supply ribose. Measurement of the pathway's activity in E. coli indicates that it is favoured during periods of growth (87)(88).

To summarize then, we can see that the examples cited above clearly reveal an apparent connection between the phosphogluconate oxidative pathway and certain synthetic processes. Efforts to ascertain the significance of this direct oxidative route, in the light of the difficulty in quantitative evaluation, have come to depend on observations of this association and on studies concerning the nature of the relationship.

The presence of the phosphogluconate oxidative pathway

in rat liver, and our present knowledge of fowl liver, suggested the probability of such a linkage existing in this avian liver. Furthermore, the onset of sexual maturity in the fowl, with the concomitant elevation of synthetic rates, presented the possibility of increased dependence on the phosphogluconate oxidative pathway when the hen reaches this physiological state.

# II. EXPERIMENTAL METHODS AND MATERIALS

## A. The Collection of Carbon Dioxide.

The measurement of $^{14}CO_2$ liberated by biological systems has received considerable attention since the earliest days of $^{14}C$ availability. As the popularity of liquid scintillation counting rapidly increased after the introduction of the Tri-Carb Liquid Scintillation Spectrometer in 1954, many workers sought to trap $^{14}CO_2$ in a medium suitable for liquid scintillation work.

The first authors to describe a method were Passman, Radin, and Cooper (89). Their trapping agent was the hydroxide of Hyamine 10-X (Reg. Trademark of Rohm & Haas, Inc.) -p-(diisobutyl-cresoxyethoxyethyl)-dimethylbenzyl-ammonium hydroxide. This hydroxide is still widely used for trapping $^{14}CO_2$ and it was so used in the experiments to be described.

Many investigators have made use of Hyamine in connection with experiments conducted in the Warburg flask. Hyamine is placed in the center well and used to trap carbon dioxide liberated from the tissue under study. Problems arise, however, in removing the Hyamine carbonate solution, or an aliquot thereof, because of the fairly viscous nature of the solution. Satisfactory

recovery of $^{14}CO_2$ requires repeated rinsing with toluene.

To overcome this difficulty, Synder and Godfrey (90) made use of a modified Warburg flask in which the central well containing the Hyamine was completely removable. When absorption of carbon dioxide was complete, the entire well was removed and dropped into a counting vial containing toluene-scintillator solution. Radioactivity was measured in a Liquid Scintillation Spectrometer and no apparent effects were noted as a result of the presence of the glass well.

The technique of Synder and Godfrey involved the modification of the Warburg vessel so that the container destined to hold the Hyamine sat atop a fixed central well. Erlenmeyer flasks (50 ml capacity), to which a central well had been fused, were used in the experiments described in this thesis. Removable wells were obtained of a diameter such that they fitted snugly inside the fused wells. The removable wells protruded above the fixed wells and could be readily and safely picked out with forceps with no danger of losing any of the Hyamine carbonate solution.

## B. The Preparation of Tissue Slices.

All experiments were performed on Single Comb White Leghorns, obtained from an inbred line developed by the Animal Science Department of Macdonald College. The birds were housed at room temperature, given an all-mash layer ration ad libitum, and allowed free access to water.

The birds were killed by cutting the jugular veins. A portion of the liver was quickly removed from the bird and placed in crushed ice. Tissue slices were cut from a one centimetre cube (approx.) of liver with a Stadie-Riggs microtome (91)(0.5mm thickness), and kept on an ice-filled covered petri dish pending transfer to the incubation flasks.

## C. The Preparation of Incubation Media.

Fifty microcurie amounts of glucose-1-$^{14}$C and of glucose-6-$^{14}$C were obtained from the Radiochemical Centre, Amersham, England. Each of the forms of specifically labeled glucose was dissolved in 3 ml of distilled water to give stock solutions containing 1.67 microcuries per 0.1 ml solution. The isotopic glucose solutions were stored in unused counting vials

and kept forzen to prevent bacterial oxidation.

Tissue slices were incubated in a Krebs-Ringer bicarbonate solution (pH 7.40-7.45), prepared as described by Umbriet (92). In all experiments, unless stated otherwise, the concentration of unlabeled glucose was 4 micromoles per ml (72mg/100 ml). The levels of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ were set as close as practicable to $3x10^5$cpm/flask. Final measurements of radioactive carbon dioxide liberation were corrected to that level on the basis of measurements of the initial incubation media. The initial levels of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ were obtained by counting aliquots of the incubation media in Bray's solution (93).

Each incubation flask contained 4ml of the medium, which had been gassed previously with $O_2$+$CO_2$(95:5) or $N_2$+$CO_2$(95:5) for ten minutes to obtain the desired pH. Stoppered flasks containing the prepared medium were stored in the refrigerator until the tissue slices were ready.

## D. Incubation and $^{14}C$ Measurement.

Incubations were carried out in triplicate or in duplicate, depending on the nature of the individual experiment. Two liver slices, weighing from 10 to 20 mg (dry weight), were placed in each flask, while two control flasks, each containing one of the forms of labeled glucose, were incubated without liver tissue.

After the tissue slices were added to the flasks and the rubber stoppers replaced, the flasks were put in an Eberbach water-bath shaker at 41.5°C. (approx. body temp. of the domestic fowl (94)). Gassing was again carried out for ten minutes with either of the two gas mixtures.

The length of time between death of the bird and the termination of the final gassing (ie. the start of the incubation period) was never greater than thirty minutes; in most experiments this time interval did not exceed 20 to 25 minutes.

Incubations were continued for sixty minutes. Five minutes before the end of the incubation period, 0.3ml of Hyamine 10-X was injected through the rubber stoppers into the removable central wells. Shaking

was resumed (100 strokes/min) until the end of the incubation period, at which time the flasks were placed in crushed ice to bring metabolism to a halt, and shaken for 45 minutes to allow absorption of the labeled carbon dioxide by the Hyamine.

The liver slices were then removed with forceps, care being taken to avoid contact between the radioactive tissue slices and the removable central well. The dry weight of the liver slices was determined after heating for two hours at 110°C. Two ml of 3N HCl was injected through the replaced rubber stoppers into the incubation media to liberate carbon dioxide from the bicarbonate solution. The flasks were then shaken for an additional 45 minutes before removal of the central wells that contained the Hyamine.

The removable wells and their contents were lifted out of the incubation flasks and dropped into counting vials containing 15 ml of scintillation fluid (0.6% PPO, 0.01% POPOP, in scintillation grade toluene). A Packard Liquid Scintillation Spectrometer was used to measure radioactivity.

## E. The Use of Estrogen *In Vivo* and *In Vitro*.

The "in vivo" effects of estrogen were studied following intramuscular injection of estradiol benzoate (Progynon-B). Ten to twelve week old pullets were given doses of one mg per day for as many days as required by the experiment.

In experiments involving the use of estrogen *in vitro*, media were prepared by the addition of aliquots from an aqueous suspension of estradiol-17 beta. The suspension was made as fine and as homogeneous as possible by the use of a Virtis homogenizer.

## F. Determination of Total Plasma Lipid.

Total plasma lipid was determined by evaporation of chloroform-methanol extracts of the plasma as described by Heald (49). Blood samples were oxalated to prevent clotting.

# III. RESULTS AND DISCUSSION.

## PART 1

### A. Duration of Incubation Period.

As noted previously, the detection of the phosphogluconate oxidative pathway by the measurement of $^{14}CO_2$ production is possible due to the earlier liberation of labeled carbon dioxide from glucose-1-$^{14}C$ when the pathway is active. Investigators of this alternate oxidative pathway have incubated the various tissues studied for different lengths of time, the range extending from one to three hours. An experiment was carried out with fowl liver slices to examine the possibility of dependency of the ratio of $^{14}CO_2$ from glucose-1-$^{14}C$ / $^{14}CO_2$ from glucose-6-$^{14}C$ upon the duration of incubation (Table I).

The variation of the ratio obtained for the different incubation times was not great and it was concluded that the length of incubation, within the time periods tested, was not a critical factor. For purposes of technique efficiency, it was decided to perform experiments on the basis of one hour incubation periods.

TABLE I.

The effect of length of incubation on the oxidation of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ to $^{14}CO_2$ by chicken liver slices.

| Incubation Time | Substrate | Oxidation of glucose to $^{14}CO_2$* $\bar{x}$ | SD $\bar{x}$ | Quotient ** |
|---|---|---|---|---|
| 30 min. | Glucose-1-$^{14}C$ | 387 | 12 | 0.92 |
| | Glucose-6-$^{14}C$ | 421 | 11 | |
| 60 min. | Glucose-1-$^{14}C$ | 1173 | 15 | 1.10 |
| | Glucose-6-$^{14}C$ | 1060 | 19 | |
| 120 min. | Glucose-1-$^{14}C$ | 3312 | 321 | 0.99 |
| | Glucose-6-$^{14}C$ | 3352 | 350 | |
| 180 min. | Glucose-1-$^{14}C$ | 4849 | 330 | 1.03 |
| | Glucose-6-$^{14}C$ | 4700 | 420 | |

* Count per minute per 10 mg dry tissue. Determinations of oxidation by individual livers performed in duplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

## B. $^{14}CO_2$ Production by Liver Slices From (1) Immature, and (2) Mature Pullets.

The measurement of the quotient, ($^{14}C$)-carbon dioxide from glucose-1-$^{14}C$ / ($^{14}C$)-carbon dioxide from glucose-6-$^{14}C$, was carried out with immature and mature pullets to examine (a) if the phosphogluconate oxidative pathway was active in the livers of both groups of pullets, and (b), to disclose whether or not there was increased activity of this pathway in the liver of pullets that had begun to lay eggs.

The results obtained with nine non-laying immature pullets (Table II) would appear to indicate that there was only a slight, though statistically significant ($P=0.01$), difference in the amount of $^{14}CO_2$ evolved from the two forms of specifically labeled glucose. A quotient of $1.02 \pm 0.06$, however, cannot be taken to suggest activity of the alternate oxidative pathway. The results, therefore, warrant the conclusion that under the "in vitro" conditions described a phosphogluconate oxidative pathway does not appear to function actively in liver slices from immature pullets.

These experiments were followed by incubations of liver slices from mature laying pullets. Quotients

were obtained for six laying pullets, and as with the values from the experiments with immature pullets, did not suggest that the tissues had metabolized glucose-1-$^{14}C$ to labeled carbon dioxide more rapidly than glucose-6-$^{14}C$ (Table III). Statistically, there was a slight excess of radioactive carbon dioxide from glucose-6-$^{14}C$ ($P=0.01$). This evidence, therefore, suggested that with mature pullets also, there is no apparent activity on the part of the phosphogluconate oxidative pathway.

The reliability of the quotient values for both groups of birds is enhanced by the fact that, despite wide variation in metabolic rate, as shown by considerable difference between individual birds in the amounts of $^{14}CO_2$ produced from the labeled glucose, the variation of the quotient was slight.

No observations based on comparison of the rates of liberation of radioactive carbon dioxide from glucose-1-$^{14}C$ and glucose-6-$^{14}C$ have been reported for hen liver in connection with the activity of the phosphogluconate oxidative pathway. The results of the above experiments were unexpected, however, for two reasons. First, values for the activity of glucose-6-phosphate dehydrogenase in hen liver have been reported previously (95), conforming with the supposition that a phosphogluconate oxidative pathway is present in the

hen liver; and secondly, the pathway is known to be active in rat liver, where it is readily detectable by the technique here employed (73)(78)(96).

To check the experimental technique, control experiments were carried out with rat liver slices (Table IV). The quotients obtained for the two rats clearly revealed an excess of $^{14}CO_2$ from glucose-1-$^{14}C$ over that oxidized from glucose-6-$^{14}C$. The results were taken as verification of the experimental method, and confirmed the presence of an active phosphogluconate pathway in rat liver as reported elsewhere (73,78,96). The quotients differed considerably from those observed with liver slices from immature and mature pullets.

The activity of the phosphogluconate oxidative pathway may be influenced by the concentration of glucose in the incubation medium. Work with mammary gland (97) and with Ehrlich ascites-tumour cells (98) has shown that the pathway may be stimulated in these tissues by increasing the medium glucose concentration. Experiments were performed, therefore, with a laying hen and an immature pullet, in which the concentration of glucose was varied from 4 to 72 microles per ml. The quotient did not appreciably exceed unity for either bird at any glucose concentration used (Table V). The oxidation of glucose by the alternate oxidative

pathway does not appear, therefore, to be dependent on the glucose concentration. Hen liver resembles rat liver in this respect (79). However, whereas with rat liver there is a proportional increase in the rates of evolution of labeled carbon dioxide from glucose-1-$^{14}C$ and glucose-6-$^{14}C$, such is not the case with hen liver, where the increments in the two rates of liberation of radioactive carbon dioxide were not proportional and the quotient decreased (Figures 1 and 2). Somewhat surprisingly, both experiments suggested a greater increase in the rate of oxidation of glucose-6-$^{14}C$ to labeled carbon dioxide at relatively higher glucose concentrations. This observation can be accepted as indicating possible activity of the uronic acid pathway at elevated glucose levels (99).

Also of interest is the fact that while 30 mM glucose is sufficient to saturate the glucose oxidizing pathways of rat liver incubated "in vitro" (79), the concentration required to achieve the same effect with hen liver is greater, even though the phosphogluconate oxidative route appears to be inactive. The two experiments with fowl liver suggest that the glucose concentration necessary to saturate the glucose oxidizing pathways may be as much as twice that needed for rat liver (Table V).

The measurement of the phosphogluconate oxidative pathway in rat liver slices involves incubation of the slices at mammalian body temperature. Since the fowl liver slices were incubated at the body temperature of the domestic fowl (41.5°C), experiments were carried out in which hen liver slices were incubated at 37.5°C. to examine the possibility that the lowering of temperature might affect the quotient. As shown in table III, the quotient remained close to unity.

Additional experiments were performed with cockerels, hens in molt, and pigeons (Table IV). The quotients for the two former groups of birds were not suggestive of an active alternate oxidation route. The incubation of pigeon liver slices produced results that do not present a clear picture. Two of the birds tested appeared to evolve a significantly greater quantity of $^{14}CO_2$ from glucose-1-$^{14}C$ than from glucose-6-$^{14}C$. The two quotients resemble in magnitude values obtained by Rossi *et al.*(80) with rat skeletal muscle, values which were taken by these authors to represent activity of the alternate oxidative route.

With the third pigeon, however, the quotient approximated unity. Further work is required, therefore, to determine a reliable value for the quotient for pigeon liver. It is of interest that in this avian

species also, the liver appears to differ considerably from rat liver if we compare activities of the phosphogluconate oxidative pathway (73,78,96).

## SUMMARY

The experiments described above indicate that the phosphogluconate oxidative pathway is not utilized to any appreciable extent by domestic fowl liver. Since rat liver possesses this alternate oxidative route, the observations with fowl liver suggest the liklihood of a significant difference in hepatic carbohydrate metabolism between these two species, and perhaps between mammal and bird.

Also of interest is the apparent failure of fowl liver to draw upon the phosphogluconate oxidative pathway when sexual maturity is reached. The involvement of the liver in the increases of the plasma constituents, and the changes in the amounts of protein and lipid in the liver itself that accompany egg production, would seemingly suggest a role for the alternate pathway during this time of greatly augmented synthetic activity. In the light of the results obtained with immature and mature pullets, it appears that if additional reduced NADP is required by the

liver, then a metabolic route other than the phospho-gluconate oxidative pathway must be called upon to meet any increased demand for this cofactor.

Figure 1.

The effect of glucose concentration on the oxidation of glucose by liver slices from an immature pullet.

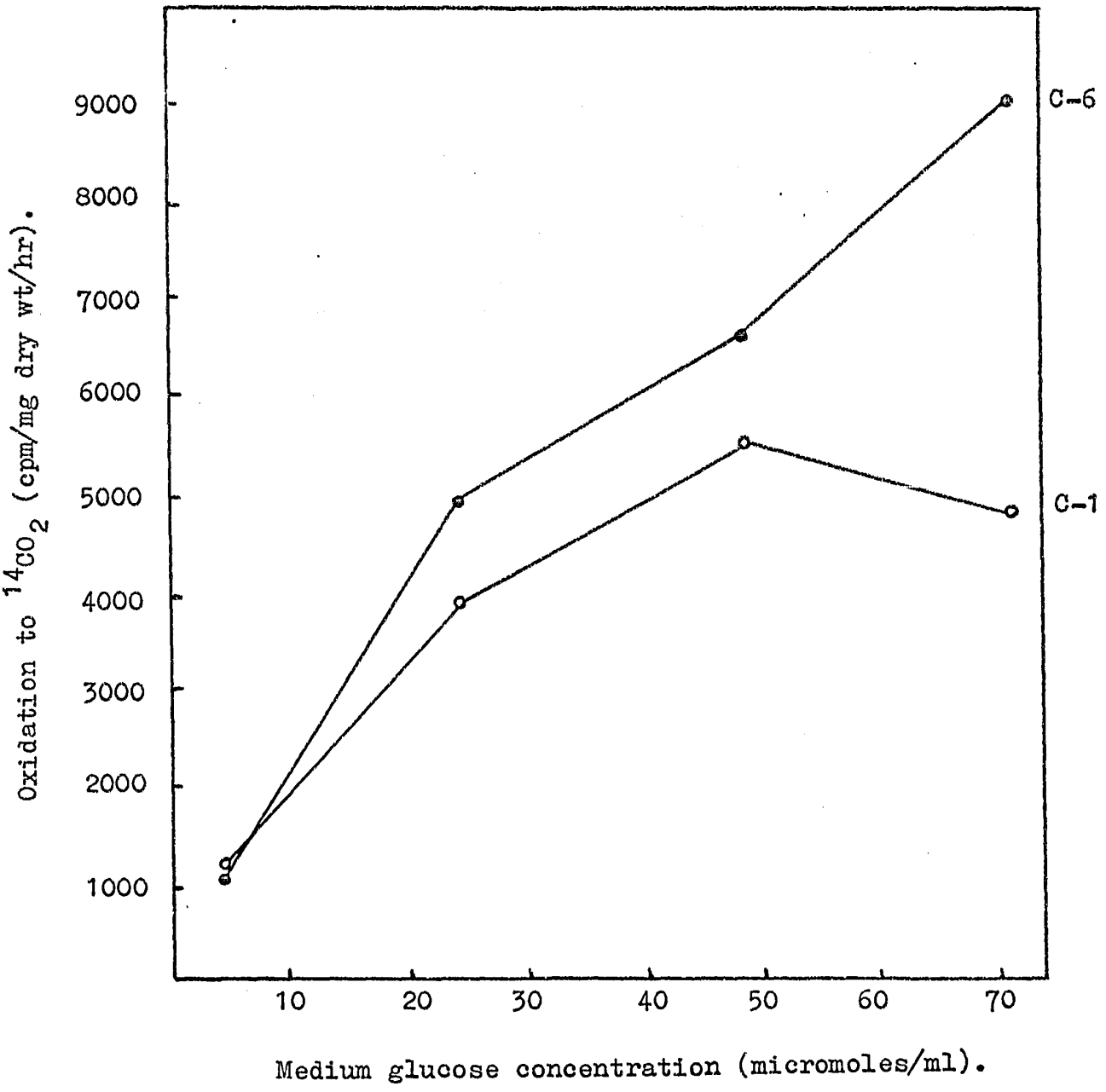

Figure 2.

The effect of glucose concentration on the oxidation of glucose by liver slices from a mature laying pullet.

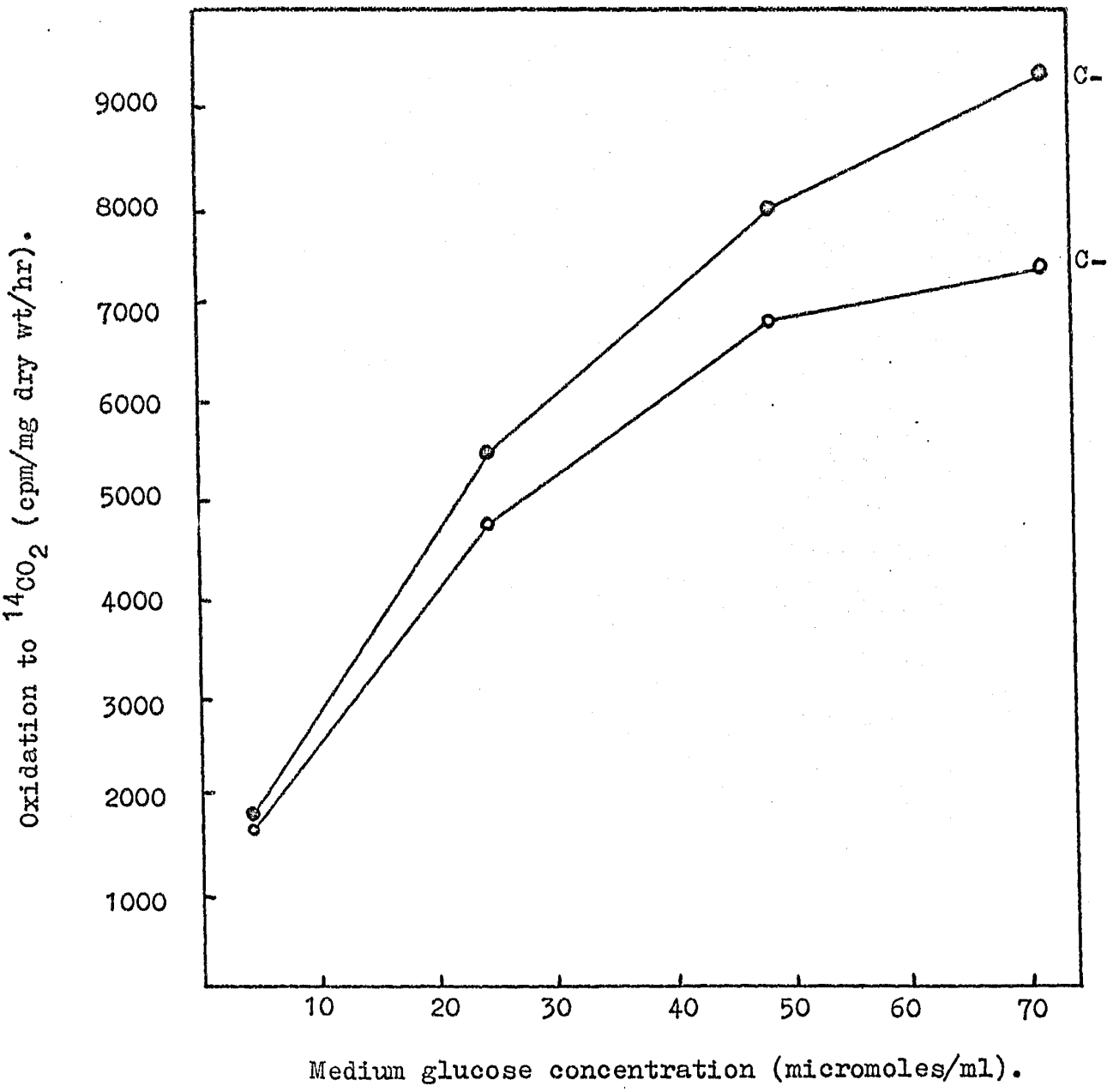

TABLE II.

The metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from immature pullets (11-12 weeks).

| Expt. No. | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | SD $\bar{x}$ | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | SD $\bar{x}$ | Quotient ** |
|---|---|---|---|---|---|
| 1 | 877 | 88 | 875 | 75 | 1.00 |
| 2 | 800 | 30 | 725 | 53 | 1.10 |
| 3 | 742 | 54 | 730 | 27 | 1.02 |
| 4 | 702 | 21 | 699 | 64 | 1.00 |
| 5 | 503 | 57 | 529 | 49 | 0.95 |
| 6 | 1003 | 8 | 937 | 65 | 1.07 |
| 7 | 468 | 11 | 505 | 14 | 0.93 |
| 8 | 988 | 19 | 984 | 57 | 1.00 |
| 9 | 606 | 10 | 551 | 19 | 1.10 |
| Means: | 743 ± 194 (SDx) | | 726 ± 177 (SDx) | | 1.02 ± 0.06 (SDx) |

*Counts per minute per 10 mg dry tissue per hour. Determinations of oxidation by individual livers were performed in triplicate.

** Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE III.

The metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from mature pullets.

| Expt. No. | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2^*$ | | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2^*$ | | Quotient ** |
|---|---|---|---|---|---|
| | $\bar{x}$ | SD $\bar{x}$ | $\bar{x}$ | SD $\bar{x}$ | |
| 1 | 1126 | 10 | 1247 | 43 | 0.90 |
| 2 | 731 | 43 | 748 | 71 | 0.98 |
| 3 | 1213 | 88 | 1143 | 46 | 1.06 |
| 4 | 578 | 36 | 700 | 45 | 0.83 |
| 5 | 422 | 36 | 504 | 54 | 0.84 |
| 6 | 564 | 75 | 601 | 111 | 0.94 |
| Means: | 772 ± 324(SDx) | | 824 ± 301(SDx) | | 0.94 ± 0.09(SDx) |
| 1 *** | 2448 | 161 | 2451 | 178 | 1.00 |
| 2 *** | 666 | 72 | 767 | 24 | 0.86 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of oxidation by individual livers were performed in triplicate.

** Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

*** Incubations of liver slices performed at 37.5°C.

TABLE IV.

The metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from (a) rats,(b) cockerels,(c) female pigeons and (d) molt hens.

| Animals | Expt. No. | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Quotient** |
|---|---|---|---|---|---|---|
| Rats | 1 | 581 | 55 | 318 | 17 | 1.83 |
| | 2 | 1186 | 121 | 670 | 50 | 1.77 |
| Cockerels | 3 | 1000 | 43 | 914 | 34 | 1.09 |
| | 4 | 1287 | 13 | 1191 | 8 | 1.08 |
| Female | 5 | 1819 | 82 | 1364 | 276 | 1.33 |
| Pigeons | 6 | 579 | 22 | 412 | 27 | 1.41 |
| | 7 | 1624 | 186 | 1396 | 140 | 1.16 |
| Molt | 8 | 1356 | 8 | 1190 | 61 | 1.14 |
| hens | 9 | 1338 | 45 | 1198 | 152 | 1.12 |
| | 10 | 2007 | 249 | 1724 | 325 | 1.16 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of oxidation by individual livers were performed in triplicate.

** Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE V.

The effect of glucose concentration on the oxidation of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ to $^{14}CO_2$ by liver slices from immature and laying pullets.

| Glucose conc. (um/ml) | Substrate | Oxidation of glucose to $^{14}CO_2$ * $\bar{x}$ | $SD_{\bar{x}}$ | Quotient ** |
|---|---|---|---|---|
| | (a) Sexually immature pullet (10 weeks) | | | |
| 4 | Glucose-1-$^{14}C$ | 1240 | 179 | 1.12 |
| | Glucose-6-$^{14}C$ | 1111 | 180 | |
| 24 | Glucose-1-$^{14}C$ | 3832 | 49 | 0.77 |
| | Glucose-6-$^{14}C$ | 4951 | (one only) | |
| 48 | Glucose-1-$^{14}C$ | 5527 | 1121 | 0.83 |
| | Glucose-6-$^{14}C$ | 6656 | 541 | |
| 72 | Glucose-1-$^{14}C$ | 4811 | 529 | 0.53 |
| | Glucose-6-$^{14}C$ | 9087 | 1576 | |
| | (b) Laying pullet | | | |
| 4 | Glucose-1-$^{14}C$ | 1680 | 16 | 0.97 |
| | Glucose-6-$^{14}C$ | 1735 | 182 | |
| 24 | Glucose-1-$^{14}C$ | 4881 | 351 | 0.88 |
| | Glucose-6-$^{14}C$ | 5571 | 726 | |
| 48 | Glucose-1-$^{14}C$ | 6827 | 291 | 0.85 |
| | Glucose-6-$^{14}C$ | 8042 | 236 | |
| 72 | Glucose-1-$^{14}C$ | 7416 | 648 | 0.79 |
| | Glucose-6-$^{14}C$ | 9379 | 276 | |

*Counts per minute per 10 mg dry tissue per hour. Determinations of oxidation by individual livers performed in duplicate.

**Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

## PART 2

In view of the results described above, further experiments were carried out in order to collect further evidence regarding the apparent relative inactivity of the phosphogluconate oxidative pathway in liver slices from the domestic fowl. Accordingly, the action of pyruvate under both aerobic and anaerobic conditions, the action of arsenite, and the effect of anaerobic incubation on labeled carbon dioxide production were examined with a view to further elucidation of the situation. The incubation of tissues under these conditions has been shown to result in elevated quotient values for tissues that possess the alternate oxidative route.

The possibility of difference between the responses of immature and mature pullets to these agents, and to the nitrogen gas phase, was also examined.

A. The Action of Arsenite on Carbon Dioxide Production.

The action of arsenite on the evolution of labeled carbon dioxide from glucose-1-$^{14}C$ and glucose-6-$^{14}C$ was tested because this compound prevents the oxidative decarboxylation of pyruvate (Peters 1953)(100). In a tissue using only the Embden-Meyerhof pathway of glucose catabolism, both C-1 and C-6 of glucose appear as the methyl group of pyruvate in equal amounts. Thus radioactive carbon dioxide derived from the Embden-Meyerhof pathway is derived equally from glucose-1-$^{14}C$ and glucose-6-$^{14}C$. Inhibition of pyruvate oxidation by arsenite should, therefore, decrease $^{14}CO_2$ production from glucose-1-$^{14}C$ and glucose-6-$^{14}C$ equally.

If the phosphogluconate oxidative pathway is active, this will not be the case. The evolution of labeled carbon dioxide from glucose-6-$^{14}C$ will be inhibited to a greater extent than that from glucose-1-$^{14}C$. An increased quotient will be observed since part of the $^{14}CO_2$ derived from glucose-1-$^{14}C$ will have arisen from reactions that precede the decarboxylation of pyruvate, i.e., it is not produced in the Krebs citric acid cycle but through the alternate oxidative route.

With immature and mature pullets, the oxidation of glucose-6-$^{14}C$ to labeled carbon dioxide was inhibited to a greater degree by arsenite than was the oxidation of glucose-1-$^{14}C$. The quotients for the two groups of fowl increased approximately fourfold, whereas the control quotients, obtained by incubation of liver slices in a medium lacking arsenite, approximated unity (Tables VI & VII).

Judging by the control quotients, it appears that the oxidation of glucose by way of the phosphogluconate oxidative pathway was stimulated by arsenite. Hoskin (101) has described a similar activation by arsenite in brain tissue slices, which normally do not possess a functioning alternate oxidative pathway; and he has suggested that arsenite action leads, in the case of brain tissue, to the utilization of an existing but ordinarily inactive pathway. This suggestion would seem to be equally applicable in explanation of the observations obtained with liver slices from the domestic fowl.

TABLE VI.

The effect of arsenite (0.3 mM) on the metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from immature pullets.

| Expt. No. | Substrate | Oxidation * control $\bar{x}$ | control $SD_{\bar{x}}$ | with arsenite $\bar{x}$ | with arsenite $SD_{\bar{x}}$ | Percent decrease with arsenite | Quotient ** control | Quotient ** with arsenite |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose-1-$^{14}C$ | 956 | 33 | 421 | 24 | 56 | 1.10 | 4.3 |
| | Glucose-6-$^{14}C$ | 872 | 76 | 98 | 4 | 89 | | |
| 2 | Glucose-1-$^{14}C$ | 616 | 59 | 316 | 20 | 49 | 0.95 | 4.1 |
| | Glucose-6-$^{14}C$ | 648 | 4 | 77 | 10 | 88 | | |
| 3 | Glucose-1-$^{14}C$ | 688 | 32 | 447 | 29 | 35 | 0.97 | 4.0 |
| | Glucose-6-$^{14}C$ | 710 | 36 | 113 | 2 | 84 | | |
| 4 | Glucose-1-$^{14}C$ | 1612 | 37 | 481 | 33 | 70 | 1.03 | 3.6 |
| | Glucose-6-$^{14}C$ | 1513 | 56 | 132 | 13 | 92 | | |

* Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in duplicate.

** Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE VII.

The effect of arsenite (0.3 mM) on the metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from laying pullets.

| Expt. No. | Substrate | Oxidation * control $\bar{x}$ | Oxidation * control $SD_{\bar{x}}$ | Oxidation * with arsenite $\bar{x}$ | Oxidation * with arsenite $SD_{\bar{x}}$ | Percent decrease with arsenite | Quotient ** control | Quotient ** with arsenite |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose-1-$^{14}C$ | 390 | 49 | 233 | 35 | 41 | 0.98 | 3.8 |
| | Glucose-6-$^{14}C$ | 395 | 122 | 61 | 12 | 85 | | |
| 2 | Glucose-1-$^{14}C$ | 1083 | 163 | 509 | 24 | 59 | 1.13 | 3.8 |
| | Glucose-6-$^{14}C$ | 957 | 174 | 134 | 4 | 86 | | |
| 3 | Glucose-1-$^{14}C$ | 1051 | 36 | 396 | 8 | 62 | 1.09 | 3.9 |
| | Glucose-6-$^{14}C$ | 968 | 11 | 101 | 4 | 90 | | |
| 4 | Glucose-1-$^{14}C$ | 1269 | 39 | 256 | 18 | 80 | 1.01 | 4.8 |
| | Glucose-6-$^{14}C$ | 1259 | 28 | 53 | 4 | 96 | | |

* Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in duplicate.

** Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

B. Incubation Under Anaerobic Conditions.

Under anaerobic conditions ($N_2$+$CO_2$-95:5), the liberation of labeled carbon dioxide from glucose-1-$^{14}C$ or from glucose-6-$^{14}C$ should be close to nil in tissues that do not possess an active phosphogluconate oxidative pathway. Should glucose be metabolized through the Embden-Meyerhof and Krebs citric acid pathways exclusively, then oxidation of glucose to carbon dioxide would not occur, because Krebs cycle does not function in the absence of oxygen. If the alternate oxidative pathway were present and active, labeled carbon dioxide would continue to be evolved from glucose-1-$^{14}C$, although in lesser amounts than would be obtained under aerobic conditions.

Hen liver slices from both immature and mature birds, when incubated anaerobically, liberated detectable quantities of radioactive carbon dioxide from the two forms of labeled glucose (Table VIII). The appearance of labeled carbox dioxide from glucose-6-$^{14}C$ when incubation was performed under a nitrogen gas phase suggests that the C-6 atom of glucose was liberated through the phosphogluconate oxidative pathway. Horecker (102) has put forward the proposal that three carbon

phosphates generated by the Embden-Meyerhof pathway may reform glucose by reversal of the aldolase reaction. It should be pointed out, however, that complete anaerobiosis may not have been achieved in the experiments with fowl liver, and incomplete anaerobiosis could also account for the appearance of some labeled carbon dioxide from glucose-6-$^{14}C$.

The experiments with liver slices taken from immature and mature fowl also revealed a slight excess of labeled carbon dioxide from glucose-1-$^{14}C$ over that recovered from glucose-6-$^{14}C$, a circumstance which again suggests that the pathway may not be totally inactive in fowl liver (Table VIII). The levels of radioactive carbon dioxide from the two types of labeled glucose were very low, however, and tend to lessen the reliability of the quotient as an indicator of activity of the alternate oxidative route, since the quotient is derived from these low values.

TABLE VIII.

The effect of pyruvate (30 mM), under anaerobic conditions, on the metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from immature and laying pullets.

| Expt. No. | Substrate | Oxidation * | | | | Quotient ** | |
|---|---|---|---|---|---|---|---|
| | | Control | | with pyruvate | | Control | with pyruvate |
| | | $\bar{x}$ | $SD_{\bar{x}}$ | $\bar{x}$ | $SD_{\bar{x}}$ | | |
| | (a) Sexually immature pullets (10-12 weeks). | | | | | | |
| 1 | Glucose-1-$^{14}C$ | 39 | 6 | 269 | 28 | 2.2 | 8.7 |
| | Glucose-6-$^{14}C$ | 18 | 4 | 31 | 4 | | |
| 2 | Glucose-1-$^{14}C$ | 67 | 3 | 445 | 9 | 1.97 | 10.9 |
| | Glucose-6-$^{14}C$ | 34 | 2 | 41 | 6 | | |
| | (b) Laying pullets. | | | | | | |
| 1 | Glucose-1-$^{14}C$ | 62 | 8 | 298 | 14 | 1.09 | 9.6 |
| | Glucose-6-$^{14}C$ | 57 | 9 | 31 | 4 | | |
| 2 | Glucose-1-$^{14}C$ | 63 | 4 | 271 | 2 | 1.40 | 8.0 |
| | Glucose-6-$^{14}C$ | 45 | 8 | 34 | 1 | | |

* Counts per minute per 10 mg dry tissue per hour. Determinations on individual livers were performed in duplicate.

** Ratio - see Table VII.

C. Action of Pyruvate Under Anaerobic Conditions.

The addition of pyruvate to the incubation medium, under anaerobic conditions, stimulated the oxidation of glucose-1-$^{14}C$ to labeled carbon dioxide, but had no evident effect on the oxidation of glucose-6-$^{14}C$ (Table VIII). Tissues incubated in the presence of 30mM pyruvate evolved eight to ten times more $^{14}CO_2$ from glucose-1-$^{14}C$ than from glucose-6-$^{14}C$. As in previous anaerobic experiments, the radioactive carbon dioxide production from glucose-6-$^{14}C$ was not reduced to nil, although the levels were very low.

Pyruvate appears, therefore, to stimulate the phosphogluconate oxidative pathway under anaerobic conditions. This activation by pyruvate can best be explained by assuming that pyruvate acts as a hydrogen-acceptor for re-oxidizing NADPH via the NADP-linked lactate dehydrogenase. Similar effects of pyruvate have been demonstrated by Kinoshita (1957)(103) in corneal epithelium, by Wenner *et al.*(1958)(104) in ascites-tumour cells, and by Beloff-Chain *et al.*(1962) (105) in the rat diaphram.

As with the demonstrated arsenite stimulation, pyruvate seems to cause activation of a pathway that

is not normally functioning in domestic fowl liver. The degree of stimulation was the same for both immature and mature pullets.

D. The Action of Pyruvate Under Aerobic Conditions.

Rossi et al.(80) have presented evidence for the existence of an active phosphogluconate oxidative pathway in skeletal muscle of rats. The evidence was based in part on observations of the action of pyruvate on the rates of oxidation of C-1 and C-6 of glucose to carbon dioxide under aerobic conditions.

It has been proposed that pyruvate, under the circumstances described, should be expected to provide isotope dilution for the Embden-Meyerhof pathway but not for the phosphogluconate oxidative pathway. This dilution would therefore lower the rate of production of $^{14}CO_2$ from glucose-6-$^{14}C$ more than from glucose-1-$^{14}C$ if the alternate oxidative route were active. Any oxidation to carbon dioxide that occurs via this pathway would not be subject to the dilution of triose phosphates by the added pyruvate, and differences in the rates of $^{14}CO_2$ liberation would therefore be magnified. If the alternate oxidative pathway is absent or non-functional, all $^{14}CO_2$ therefore being liberated through the Krebs

Cycle, then the amounts of labeled carbon dioxide evolved from glucose-1-$^{14}C$ and glucose-6-$^{14}C$ should be equal since both forms of the isotope are degraded to pyruvate labeled at the methyl group.

Hen liver slices from immature and mature birds revealed a greater decrease in labeled carbon dioxide production from glucose-6-$^{14}C$ than from glucose-1-$^{14}C$ (Tables IX & X). With all hens observed, the quotient increased approximately threefold and therefore indicated activity of the phosphogluconate oxidative pathway in the presence of pyruvate and an aerobic gas phase. The control quotients were close to unity, however, indicating inactivity of the pathway. These observations with fowl liver suggest that pyruvate stimulates the alternate oxidative route when incubation is carried out under an $O_2$+$CO_2$(95:5) gas phase. Pyruvate appears, therefore, to activate the phosphogluconate oxidative pathway under aerobic as well as under anaerobic conditions.

A comparison of the control and pyruvate quotients points out a possible fallacy in conclusions based on this particular experiment. It has been thought that an increase in the quotient with pyruvate, under aerobic conditions, may be taken as supporting evidence

TABLE IX

The effect of pyruvate (30 mM), under aerobic conditions, on the metabolism of glucose-1-$^{14}$C and glucose-6-$^{14}$C by liver slices from immature pullets.

| Expt. No. | Substrate | Oxidation* control $\bar{x}$ | Oxidation* control $SD_{\bar{x}}$ | Oxidation* with pyruvate $\bar{x}$ | Oxidation* with pyruvate $SD_{\bar{x}}$ | Percent decrease with pyruvate | Quotient** control | Quotient** with pyruvate |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose-1-$^{14}$C | 2060 | 294 | 324 | 26 | 84 | 1.01 | 3.3 |
| | Glucose-6-$^{14}$C | 2035 | 178 | 98 | 3 | 95 | | |
| 2 | Glucose-1-$^{14}$C | 1423 | 41 | 432 | 5 | 70 | 0.89 | 3.2 |
| | Glucose-6-$^{14}$C | 1598 | 86 | 137 | 2 | 91 | | |
| 3 | Glucose-1-$^{14}$C | 1260 | 103 | 301 | 9 | 76 | 1.03 | 2.9 |
| | Glucose-6-$^{14}$C | 1220 | 47 | 104 | 4 | 92 | | |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in duplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE X

The effect of pyruvate (30 mM), under aerobic conditions, on the metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from laying pullets.

| Expt. No. | Substrate | Oxidation * control $\bar{x}$ | Oxidation * control $SD_{\bar{x}}$ | Oxidation * with pyruvate $\bar{x}$ | Oxidation * with pyruvate $SD_{\bar{x}}$ | Percent decrease with pyruvate | Quotient** control | Quotient** with pyruvate |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose-1-$^{14}C$ | 936 | 53 | 327 | 19 | 65 | 0.90 | 2.8 |
| | Glucose-6-$^{14}C$ | 1038 | 115 | 119 | 16 | 89 | | |
| 2 | Glucose-1-$^{14}C$ | 1007 | 35 | 213 | 16 | 79 | 1.02 | 3.0 |
| | Glucose-6-$^{14}C$ | 990 | 13 | 71 | 2 | 93 | | |
| 3 | Glucose-1-$^{14}C$ | 732 | 15 | 190 | 1 | 74 | 0.92 | 4.4 |
| | Glucose-6-$^{14}C$ | 794 | (one only) | 43 | 1 | 95 | | |

* Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in duplicate.

** Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

in favour of the presence of an operating phosphogluconate oxidative pathway. However, if the control quotient is not sufficiently great to establish clearly the existence of the pathway, then the alleged supporting evidence may in fact simply indicate stimulation under physiologically abnormal conditions (i.e. 30mM pyruvate) of a normally non-functional phosphogluconate oxidative pathway.

## SUMMARY

An examination of the quotient values obtained in the absence of either arsenite or pyruvate, and those obtained in the presence of these two compounds, permits the conclusion that although the phosphogluconate oxidative pathway is apparently inactive in fowl liver slices incubated with glucose, "in vitro", it is possible to stimulate the pathway. The ratios obtained following anaerobic incubation with labeled glucose suggest that the pathway in fowl liver is perhaps slightly active and confirms the view that the activity, if indeed real, is in no way comparable in degree with that found in rat liver.

It was also noted that liver slices from immature and mature pullets responded similarly to pyruvate, arsenite, and an anaerobic gas phase, the observations indicating no marked variation in the contribution by the phosphogluconate oxidative pathway to glucose oxidation, under the experimental conditions utilized, as between the two groups of birds.

## PART 3

### A. Activity of the Phosphogluconate Oxidative Pathway in the Liver of Pullets Approaching Sexual Maturity.

Heald and Badman (49) have shown that the levels of plasma free fatty acids, total lipid, and phospholipoprotein display marked increases as maturing pullets approach laying. These levels drop sharply once laying has commenced, although they remain higher than the levels of these plasma components found in immature pullets.

Although the evidence presented in previous sections of this thesis failed to establish the existence of an active phosphogluconate oxidative pathway in fowl liver, there remained the possibility that the pathway may become functional during the two to three week period prior to the laying of the first egg. It is during this period that the plasma components mentioned above reach maximal levels, as does also the total protein level.

To examine the possibility that the alternate oxidative route might be active at this time when the

puberal hen comes into lay, a group of birds was followed through the ages of 15 to 21 weeks. Oviduct weights and total plasma lipid were recorded as indices of the onset of sexual maturity for each bird (Tables - pages 64-69).

The data provide no evidence for the activation of the phosphogluconate oxidative pathway in tandem with the elevation of total plasma lipid, or with the increase in oviduct weight. The quotient values for 15 to 17 week old pullets indicate the possibility of the pathway having become slightly active in some birds around the time when hypertrophy of the oviduct was in its initial stages. However, the semiquantitative nature of the quotient requires that this interpretation be weighed accordingly. In experiments with birds in this age group, the quotient values were considerably lower than those obtained with rat liver, and very much lower than the values reported for other mammalian tissues such as adrenal cortex (85) and lactating mammary gland (106).

This apparent slight activity of the alternate route in the livers of some birds aged 15 to 17 weeks, if real, was not detected in pullets over 17 weeks of age. As the observations reveal, it was from the 18th week onward that the increases in oviduct weight

and total plasma lipid occured.

The data warrant the conclusion that the phosphogluconate oxidative pathway in the liver of birds approaching sexual maturity does not appear to become active, with the possible exception of the period when oviduct hypertrophy begins. The evidence does not suggest that the attainment of the excessively high levels of plasma total lipid requires the co-operation of the alternate oxidative pathway.

### B. The Effect of "In Vivo" Pretreatment of Immature Pullets with Estrogen.

The administration of estrogen to rats has been shown to accelerate the phosphogluconate oxidative pathway in liver slices (106) subsequently obtained from the animals. The response of immature pullets to such treatment is well documented and, as mentioned previously, involves the elevation of the levels of plasma lipid and protein, as well as elevation of the amounts of lipid and protein in the liver.

Estradiol monobenzoate (1 mg/day) was injected into immature pullets for periods of one to ten days. The expected increases in oviduct weight and total plasma lipid were observed, but they were not accompanied

by increased liberation of labeled carbon dioxide from glucose-1-$^{14}C$, relative to that evolved from glucose-6-$^{14}C$ (Tables XV & XVI). "In vivo" pretreatment with this estrogen, therefore, does not appear to influence the activity of the alternate oxidative pathway in hen liver slices incubated "in vitro". These observations with immature pullets are in accordance with the quotient values obtained with fowl coming into lay normally.

Again, the results bring out the fact that increased requirement for NADPH is apparently not satisfied by the phosphogluconate oxidative pathway. Furthermore, the difference between rat liver and domestic fowl liver in the manner in which glucose is oxidized, is made obvious by a comparison of the results for these fowl with recorded observations of the effect of estrogen on rat liver (106).

TABLE XI.

Metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from pullets over the period of sexual immaturity to full reproductive activity.

A. 15 week old pullets.

| Expt. No. | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2^*$ | | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2^*$ | | Quotient** |
|---|---|---|---|---|---|---|---|
| | | | $\bar{x}$ | $SD_{\bar{x}}$ | $\bar{x}$ | $SD_{\bar{x}}$ | |
| 1 | - | - | 1577 | 137 | 1275 | 73 | 1.24 |
| 2 | - | - | 2152 | 107 | 1905 | 137 | 1.13 |
| 3 | - | - | 1944 | 110 | 1545 | 144 | 1.26 |
| 4 | 0.68 | 0.60 | 1612 | 39 | 1332 | 136 | 1.21 |
| 5 | 0.31 | 0.64 | 1653 | 83 | 1415 | 61 | 1.17 |
| 6 | 0.77 | 0.60 | 793 | 98 | 659 | 81 | 1.20 |
| 7 | 0.78 | 0.60 | 1013 | 34 | 956 | 23 | 1.06 |
| 8 | 0.23 | 0.52 | 1730 | 78 | 1635 | 31 | 1.06 |
| Means: ($\pm$SDx) | 0.55$\pm$0.27 | 0.59$\pm$0.04 | 1559$\pm$451 | | 1340$\pm$391 | | 1.17$\pm$0.08 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XI (contd.)

B. 16 week old pullets.

| Expt. No. | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Quotient** |
|---|---|---|---|---|---|---|---|
| 1 | 0.34 | 0.56 | 1187 | 81 | 945 | 85 | 1.26 |
| 2 | 2.2 | 0.62 | 1369 | 60 | 1147 | 50 | 1.19 |
| 3 | 8.8 | 0.68 | 940 | 75 | 787 | 44 | 1.19 |
| 4 | 6.4 | 0.62 | 1600 | 129 | 1567 | 117 | 1.02 |
| Means: ($\pm$SDx) | 4.4$\pm$3.9 | 0.62$\pm$0.05 | 1274$\pm$279 | | 1112$\pm$338 | | 1.16$\pm$0.1 |

* Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XI (contd.)

C. 17 and 18 week old pullets.

| Expt. No. | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Quotient** |
|---|---|---|---|---|---|---|---|
| | | | 17 week old pullets. | | | | |
| 1 | - | - | 1780 | 133 | 1464 | 132 | 1.22 |
| 2 | 1.4 | 0.7 | 1433 | 214 | 1330 | 99 | 1.08 |
| Means: (±SDx) | 1.4 | 0.7 | 1607±245 | | 1397±95 | | 1.15±0.1 |
| | | | 18 week old pullets. | | | | |
| 1 | 4.4 | 0.51 | 1106 | 38 | 1112 | 46 | 1.00 |
| 2 | 0.46 | 0.52 | 1830 | 156 | 1996 | 109 | 0.92 |
| 3 | 7.4 | 0.42 | 966 | 54 | 964 | 35 | 1.00 |
| Means: (±SDx) | 4.1±3.5 | 0.48±0.06 | 1301±464 | | 1357±558 | | 0.97±0.07 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XI (contd.)

D. 19 week old pullets.

| Expt. No. | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* $\bar{x}$ | $SD_{\bar{x}}$ | Quotient** |
|---|---|---|---|---|---|---|---|
| 1 | 0.41 | 0.58 | 793 | 40 | 787 | 14 | 1.01 |
| 2 | 2.6 | 0.52 | 1318 | 154 | 1289 | 64 | 1.02 |
| 3 | 5.0 | 0.86 | 1213 | 72 | 1158 | 46 | 1.05 |
| 4 | 1.6 | 0.72 | 2099 | 94 | 1786 | 99 | 1.18 |
| 5 | 14.0 | 0.94 | 1834 | 95 | 1688 | 49 | 1.09 |
| 6 | 13.0 | 0.84 | 1619 | 76 | 1409 | 64 | 1.15 |
| 7 | 25.2 | 2.44 | 1184 | 100 | 1342 | 91 | 0.88 |
| Means: ($\pm$SDx) | 8.8$\pm$9.0 | 0.99$\pm$0.66 | 1437$\pm$442 | | 1351$\pm$333 | | 1.05$\pm$0.1 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XI (contd.)

E. 20 week old pullets.

| Expt. No. | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* | | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* | | Quotient** |
|---|---|---|---|---|---|---|---|
| | | | $\bar{x}$ | $SD_{\bar{x}}$ | $\bar{x}$ | $SD_{\bar{x}}$ | |
| 1 | 21.0 | 1.26 | 1704 | 133 | 1608 | 60 | 1.06 |
| 2 | 20.9 | 1.38 | 1224 | 43 | 1070 | 116 | 1.14 |
| 3 | 27.3 | 5.32 | 931 | 14 | 906 | 14 | 1.03 |
| 4 | 12.0 | 0.90 | 2420 | 145 | 2264 | 262 | 1.07 |
| 5 | 17.1 | 0.84 | 1584 | 40 | 1572 | 114 | 1.01 |
| 6 | 36.4 | 2.66 | 915 | 88 | 1048 | 28 | 0.87 |
| Means: ($\pm$SDx) | 22.5$\pm$8.4 | 2.06$\pm$1.72 | 1463$\pm$577 | | 1411$\pm$509 | | 1.03$\pm$0.09 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XI (contd.)

F. 21 week old pullets.

| Expt. No. | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$* | | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* | | Quotient** |
|---|---|---|---|---|---|---|---|
| | | | $\bar{x}$ | $SD_{\bar{x}}$ | $\bar{x}$ | $SD_{\bar{x}}$ | |
| 1 | 14.7 | 0.78 | 1565 | 71 | 1412 | 50 | 1.11 |
| 2 | 8.8 | 0.64 | 2194 | 203 | 2090 | 131 | 1.05 |
| 3 | 8.2 | 0.94 | 836 | 85 | 720 | 34 | 1.16 |
| 4 | 33.1 | 2.60 | 1659 | 127 | 1544 | 148 | 1.07 |
| 5 | 20.4 | 1.26 | 700 | 1 | 665 | 77 | 1.05 |
| Means: (±SDx) | 17.0±10.3 | 1.24±0.79 | 1391±618 | | 1286±598 | | 1.09±0.05 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XII.

The effect of "in vivo" pretreatment with estradiol-17beta mono benzoate on the metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from sexually immature pullets (10-11 weeks).

| Expt. No. | Estradiol 1 mg/day | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$ | | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2^*$ | | Quotient** |
|---|---|---|---|---|---|---|---|---|
| | | | | $\bar{x}$ | $SD_{\bar{x}}$ | $\bar{x}$ | $SD_{\bar{x}}$ | |
| 1 | 1 day | 0.55 | 0.82 | 1920 | 174 | 2223 | 53 | 0.86 |
| 2 | 1 day | 0.39 | 1.0 | 1562 | 55 | 1349 | 86 | 1.16 |
| 3 | 2 days | 1.1 | 1.2 | 1249 | 24 | 1156 | 19 | 1.08 |
| 4 | 2 days | 0.8 | 0.8 | 741 | 40 | 616 | 54 | 1.20 |
| 5 | 4 days | 4.1 | 2.1 | 1664 | 111 | 1524 | 57 | 1.09 |
| 6 | 4 days | 4.8 | 1.4 | 1593 | 36 | 1667 | 62 | 0.96 |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

**Ratio $\frac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

TABLE XII.(contd.)

| Expt. No. | Estradiol 1 mg/day | Oviduct wt. (g) | Plasma lipid g/100 ml | Oxidation of glucose-1-$^{14}C$ to $^{14}CO_2$ | | Oxidation of glucose-6-$^{14}C$ to $^{14}CO_2$* | | Quotient ** |
|---|---|---|---|---|---|---|---|---|
| | | | | $\bar{x}$ | $SD_{\bar{x}}$ | $\bar{x}$ | $SD_{\bar{x}}$ | |
| 7 | 6 days | 7.3 | 2.8 | 1701 | 73 | 1578 | 101 | 1.08 |
| 8 | 6 days | 6.3 | 4.4 | 2379 | 196 | 2327 | 154 | 1.02 |
| 9 | 8 days | 8.1 | 5.2 | 733 | 156 | 779 | 24 | 0.94 |
| 10 | 8 days | 8.5 | 5.8 | 1400 | 77 | 1389 | 128 | 1.01 |
| 11 | 10 days | 10.8 | 10.0 | 705 | 45 | 550 | 20 | 1.28 |
| 12 | 10 days | 12.5 | 9.0 | 1623 | 60 | 1477 | 10 | 1.10 |

* Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in triplicate.

** Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

### C. The "In Vitro" Effects of Estrogen on the Liberation of $^{14}CO_2$ by Liver Slices from Immature Pullets.

Experiments were carried out to examine the effect of "in vitro" addition of estradiol-17beta on the liberation of labeled carbon dioxide from glucose-1-$^{14}$C and glucose-6-$^{14}$C. The incubation of hen liver slices in incubation media containing either 2 or 20 micrograms of estradiol-17beta per ml, did not reveal activation of the phosphogluconate oxidative pathway (Table XVII). The rates of liberation of $^{14}CO_2$ from glucose-1-$^{14}$C and glucose-6-$^{14}$C were practically the same, the quotients approximating unity. There was no significant difference between the estradiol-17beta quotient and the control quotient.

The results of these experiments are not surprising in view of the observations, as reported by Heald (1965) (107), of the "in vitro" effect of estradiol-17beta on the synthesis of phosvitin from (2-$^{14}$C)-glycine, inasmuch as the addition of estrogen to slices from immature birds did not promote protein synthesis. This inability of estrogen to increase phosvitin synthesis, when added "in vitro", suggested either that the action of estrogen "in vivo" is an indirect one, or that the period of

incubation used "in vitro" was too short for any such action to become apparent. Furthermore, none of the experiments described in this thesis that had been carried out up to this point had given cause to suspect estrogen activation of the alternate oxidative pathway.

Estradiol-17beta, "in vitro", therefore does appear not to stimulate the phosphogluconate oxidative pathway in liver slices from the immature domestic fowl.

TABLE XIII.

The effect of estradiol-17beta, "*in vitro*", on the metabolism of glucose-1-$^{14}C$ and glucose-6-$^{14}C$ by liver slices from immature pullets (12-13 weeks).

| Expt. No. | Substrate | Estradiol-17beta microgram/ml | Oxidation * control $\bar{x}$ | Oxidation * control $SD_{\bar{x}}$ | Oxidation * estradiol-17beta $\bar{x}$ | Oxidation * estradiol-17beta $SD_{\bar{x}}$ | Quotient ** control | Quotient ** estradiol-17beta |
|---|---|---|---|---|---|---|---|---|
| 1 | Glucose-1-$^{14}C$ | 2 | 857 | 25 | 774 | 122 | 1.14 | 1.05 |
| | Glucose-6-$^{14}C$ | | 752 | 45 | 734 | 32 | | |
| 2 | Glucose-1-$^{14}C$ | 2 | 1786 | 61 | 1507 | 330 | 1.02 | 0.96 |
| | Glucose-6-$^{14}C$ | | 1759 | 34 | 1571 | 3 | | |
| 3 | Glucose-1-$^{14}C$ | 20 | 1657 | 2 | 1560 | 50 | 1.16 | 1.04 |
| | Glucose-6-$^{14}C$ | | 1432 | 33 | 1496 | 91 | | |
| 4 | Glucose-1-$^{14}C$ | 20 | 2216 | 216 | 2070 | 13 | 0.99 | 1.18 |
| | Glucose-6-$^{14}C$ | | 2250 | 71 | 1749 | 175 | | |

*Counts per minute per 10 mg dry tissue per hour. Determinations of glucose oxidation by individual livers performed in duplicate.

** Ratio $\dfrac{^{14}CO_2 \text{ from glucose-1-}^{14}C}{^{14}CO_2 \text{ from glucose-6-}^{14}C}$

## IV. CONCLUSIONS

1. Measurement of the rates of liberation of labeled carbon dioxide from glucose-1-$^{14}C$ and glucose-6-$^{14}C$ indicate that the phosphogluconate oxidative pathway does not normally function in the livers of immature and mature pullets.

2. The ratio, $^{14}CO_2$ from glucose-1-$^{14}C$: $^{14}CO_2$ from glucose-6-$^{14}C$, was not influenced by the length of time incubations were carried out, nor was it affected by incubating fowl liver slices at mammalian body temperature.

3. The activity of the phosphogluconate oxidative pathway was shown not to be influenced by the concentration of glucose in the incubation medium. The quotient did not appreciably exceed unity at any glucose concentration tested. At high glucose concentrations there was a greater increase in the rate of oxidation of glucose-6-$^{14}C$ to labeled carbon dioxide, indicating possible activity of the uronic acid pathway at elevated glucose levels.

4. The glucose concentration required to saturate the glucose oxidizing pathways was shown to be approximately double that needed for rat liver.

5. It was also found that the phosphogluconate oxidative pathway is apparently inactive in cockerels and molt hens. Work carried out on pigeons suggests that in this avian species also, the liver appears to differ considerably from rat liver when comparing activities of the phosphogluconate oxidative pathway.

6. The addition of arsenite to the incubation medium stimulated the phosphogluconate oxidative pathway in liver slices from immature and mature pullets.

7. The incubation of liver slices from immature and mature pullets under anaerobic conditions resulted in marked reductions in the liberation of labeled carbon dioxide from the two forms of specifically labeled glucose. There was a slight excess of radioactive carbon dioxide from glucose-1-$^{14}C$ which suggests that the phosphogluconate oxidative pathway may not be totally inactive in fowl liver.

8. The addition of pyruvate to the incubation medium, under anaerobic conditions, stimulated the phosphogluconate oxidative pathway in liver slices from immature and mature pullets.

9. Pyruvate also stimulated the phosphogluconate oxidative pathway in liver slices from immature and mature pullets when incubation was carried out under

aerobic conditions.

10. The phosphogluconate oxidative pathway in the liver of pullets approaching sexual maturity was found not to become active in tandem with the elevation of total plasma lipid, nor with the increase in oviduct weight.

11. The administration of estradiol monobenzoate to immature pullets was shown not to accelerate the phosphogluconate oxidative pathway.

12. It was also found that the addition of estradiol-17 beta to the incubation medium, does not stimulate the phosphogluconate oxidative pathway in liver slices from the immature domestic fowl.

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