strong than for ribose 5-phosphate and sedoheptulose 7-phosphate in that the sugar moiety was not recovered after hydrolysis: this is likely to be due to the very small amount of this compound present in skin. We thus conclude that the hexose monophosphate pathway operates in skin.

SUMMARY

1. Skin slices were incubated in a medium containing uniformly labelled [^{14}C]glucose or [^{32}P]orthophosphate or both. The phosphate esters present in a trichloroacetic acid extract of such skin were separated by two-dimensional chromatography.

2. Of the labelled phosphates present, sedoheptulose 7-phosphate, sedoheptulose diphosphate and ribose 5-phosphate were identified by co-chromatography.

3. The identity of sedoheptulose 7-phosphate and ribose 5-phosphate was confirmed by co-chromatography of their labelled hydrolysis products with the appropriate sugar.

4. The demonstration of these compounds in guinea-pig-ear skin provides direct evidence that the hexose monophosphate pathway operates in this tissue.

Our thanks are due to Dr C. N. D. Cruickshank for his valuable advice and criticism.

REFERENCES

- Barron, E. S. G., Meyer, J. & Miller, Z. B. (1948). *J. invest. Derm.* **11**, 97.
 Bevenue, A. & Williams, K. T. (1960). *J. Chromat.* **4**, 391.
 Brooks, S. A., Lawrence, J. C. & Ricketts, C. R. (1959). *Biochem. J.* **73**, 566.
 Burrows, S., Grylls, F. S. M. & Harrison, J. S. (1952). *Nature, Lond.*, **170**, 800.
 Freinkel, R. K. (1960). *J. invest. Derm.* **34**, 37.
 Gilbert, D. (1962). *J. invest. Derm.* **38**, 123.
 Hershey, F. B., Lewis, C., Murphy, J. & Schiff, T. (1960). *J. Histochem. Cytochem.* **8**, 41.
 Hershey, F. B. & Mendle, B. J. (1954). *Surg. Forum*, **5**, 745.
 Katz, J. & Wood, H. G. (1960). *J. biol. Chem.* **235**, 2165.
 Krebs, H. A. (1933). *Hoppe-Seyl. Z.* **217**, 191.
 Moses, V. (1960). In *Chromatographic and Electrophoretic Techniques*, vol. 1, p. 527. Ed. by Smith, I. London: William Heinemann Ltd.
 Olley, J. & Dawson, R. M. C. (1956). *Biochem. J.* **62**, 5 p.
 Partridge, S. M. (1949). *Nature, Lond.*, **164**, 443.
 Pomerantz, S. H. & Asbornsen, M. T. (1961). *Arch. Biochem. Biophys.* **93**, 147.
 Wawszkiewicz, E. J. (1961). *Analyt. Chem.* **33**, 252.
 Wood, H. G. (1955). *Physiol. Rev.* **35**, 841.
 Wood, T. (1961). *J. Chromat.* **6**, 142.

Biochem. J. (1963) **86**, 103

The Metabolism of Carbohydrate by Liver of the Domestic Fowl

By P. J. HEALD

Twyford Laboratories Ltd., Twyford Abbey Road, London, N.W. 10

(Received 27 June 1962)

Although there exists a considerable literature on the carbohydrate metabolism of mammalian liver, by contrast that relating to avian liver is relatively small. 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. Information on the carbohydrate metabolism of the liver, and its ability or otherwise to use various carbohydrates as substrates under these two conditions, has not been recorded. The present experiments were therefore undertaken as part of a study of the metabolism of the liver of the domestic fowl as it is affected by the onset of laying.

MATERIALS AND METHODS

Birds. The domestic fowls were Single Comb White Leghorns of an inbred line purchased from Moncrieff's Animal Station, Godstone, Surrey. They were maintained

under artificial daylight at a constant temperature of 60–62° F. Birds in lay were exposed alternately to 14 hr. of light from 6 a.m. to 8 p.m. followed by 10 hr. of darkness. Birds not in lay were exposed alternately to 10 hr. of light from 8 a.m. to 6 p.m. followed by 14 hr. of darkness. Moulting was induced in a few birds by decreasing the light to a minimum of 6 hr./day. This was not uniformly successful, some birds continuing to lay with an unimpaired performance. The birds were fed either on 'Intensive growers pellets' or on 'Battery layers pellets' (British Oil and Cake Mills Ltd.) *ad libitum* as required and were allowed free access to water.

Pigeons were obtained from O. Kershaw, 22 Hope Street, Manor Road, Ossett, Yorkshire, and were maintained on a diet of corn, maple peas and limestone grit. The physiological state of the birds, i.e. in lay or not in lay, was determined by daily egg records, by the presence or absence of lipophosphoprotein in the plasma (McIndoe, 1959) and by examination of the ovaries at autopsy.

Preparation of tissue slices. Birds were killed by rapidly severing the neck vessels and were allowed to bleed as much as possible. The liver was rapidly excised and placed

in a large Petri dish containing a filter paper moistened with saline (see below). Tissue slices were cut with a razor blade with a guide as described by McIlwain (1951). Slices were floated into saline, trimmed with a scalpel, drained against a clock glass and weighed rapidly on a torsion balance before being placed in the manometric vessels. Generally less emphasis was placed on careful draining and weighing than on getting the tissue into the incubation medium quickly. The liver of the laying hen contains much fat, some of which is leached out by the medium both during slicing and subsequent incubation. For this reason calculations were made on the basis of the dry weight of the intact tissue slice after incubation, this being more accurately representative of the metabolically active material.

Saline solutions. The saline solutions were the phosphate- and bicarbonate-buffered saline solutions described by Krebs & Henseleit (1932). Substrates were included to give a final concentration of 10 mM.

Manometry. Oxygen uptake in phosphate-buffered saline solutions and aerobic gas exchange in bicarbonate-buffered saline solutions were determined with conventional Warburg vessels with a single side arm. Vessels contained 1.5 ml. of medium and 60–70 mg. wet wt. of liver tissue. Oxygen uptake in bicarbonate-buffered saline and the respiratory quotient, $R.Q.$, were determined in the vessels described by Dickens & Simer (1931), with the technique described by those authors without modification. In these experiments the tissue added was 50–60 mg. wet wt. Since the body temperature of the domestic fowl is 41.2 – 41.6° (Sturkie, 1954) all measurements were made at 41.3° except where stated otherwise. At the end of the incubation period the slices were briefly rinsed in water before the dry wt. was determined by heating at 110° for 18 hr.

Analytical methods. Glucose was estimated manometrically with glucose oxidase (Worthington Biochemical Corp., Freehold, N.J., U.S.A.) (Keilin & Hartree, 1948). The enzyme (10 mg.) was added from the side arm. Catalase (0.7 mg./vessel) was obtained from Biochimica Boehringer (Mannheim, Germany).

Fructose was estimated by the method of Roe (1934) as described by LePage (1957) in solutions deproteinized with $ZnSO_4$ -NaOH reagent as described by Mann (1946).

Glycogen was determined as described by Hassid & Abraham (1957).

Lactic acid was determined by the method of Barker & Summerson (1941).

All substrates were either analytical grade or bacteriological sugars.

The metabolic quotients were defined as follows: $-Q_{O_2}$, μ l. of O_2 consumed/mg. dry wt. of tissue/hr.; Q_{acid} , μ l. of gas (released by acid production)/mg. dry wt. of tissue/hr.; $-q_{substrate}$, mg. of substrate consumed/mg. dry wt. of tissue/hr.

RESULTS

Respiration with added substrates. The oxygen uptake of slices of liver in a medium buffered with phosphate was markedly increased in the presence of fructose (Fig. 1). This increase occurred with tissue from birds aged 5–48 weeks and in both cockerels and pullets. The increase was not affected by starving the birds for 18–24 hr. (which lowered the initial glycogen content of the slices

from 13–15 % to 0.25–1.0 %), although the endogenous oxygen uptake was markedly reduced. The increased oxygen uptake was usually linear and continued for at least 3 hr., the maximum period examined. Incubation in the absence of fructose reduced the activity of the oxidizing system (Fig. 1), since fructose added after preincubation for 1 hr. did not result in an increased oxygen uptake equal to that when fructose was present from the start.

A similar increase was obtained with L(-)-sorbitol but not with D(-)-tagatose. Other sugars not supporting oxygen uptake in this medium were glucose (at 10 or 100 mM), galactose, mannose, mannitol, dulcitol, sorbitol, ribose and glucosamine. Glucosamine at concentrations higher than 10 mM inhibited oxygen uptake. Oxygen uptake was increased by pyruvate, fumarate, succinate and glutamate, but not by citrate, α -oxoglutarate, malate, β -hydroxybutyrate, glycerol, β -glycerophosphate and 2- and 3-phosphoglycerates.

The inability of sorbitol to support respiration is in contrast with its effect on slices of rat liver, by which it is metabolized with an increased oxygen uptake (Blakley, 1951). It was therefore of interest to see whether fowl liver contained sorbitol dehydrogenase. The results of such experiments

Fig. 1. Oxygen uptake of slices of fowl liver in the presence of fructose. The bird, aged 9.0 weeks, was fed. Incubation was in phosphate-buffered saline at 41.3° . The gas phase was oxygen. ○, With fructose (10 mM); ●, without substrate or with glucose (10 mM); ⊙, fructose (final concn. 10 mM) added at A to slices incubated in the absence of fructose.

(Table 1) showed that sorbitol was readily oxidized by aqueous dispersions of fowl liver. In the same Table, as a contrast, is listed the oxygen uptake of slices of liver with sorbitol as a substrate. Since a sorbitol dehydrogenase is clearly present in the tissues the failure of sorbitol to increase the oxygen uptake of slices is presumably due to failure of the substrate to enter the tissue.

In a medium buffered with bicarbonate the oxygen uptake in the presence of fructose was not markedly different from that in a medium buffered with phosphate. Thus in two experiments in which the oxygen uptake of liver slices was measured simultaneously in the presence of both buffers the

Q_{O_2} values were: bicarbonate, -14.8 , and phosphate, -14.1 ; and bicarbonate, -13.3 , and phosphate -13.2 .

Effect of temperature. The temperature of incubation of the fowl-liver slices was 41.3° . Slices of mammalian liver do not oxidize fructose with an increased oxygen uptake at 37.5° (cf. Dickens & Greville, 1933). However, at this lower temperature the increased oxygen uptake of slices of fowl liver in the presence of fructose was still clearly observed (Table 2). At neither temperature was the oxygen uptake of slices of rat liver increased by fructose. With starved animals the uptake of fructose by rat liver was less than with fowl liver

Table 1. *Oxygen uptake of slices and of aqueous dispersions of fowl liver in the presence and absence of sorbitol*

Slices were cut and incubated in phosphate-buffered saline for 3 hr. at 41.3° as described in the Materials and Methods section. Dispersions (1:10, w/v) in 0.01M-potassium phosphate buffer, pH 7.6, were incubated in the following system (cf. Blakley, 1951): dispersion (0.5 ml.), 0.1M-KCl (0.3 ml.), 0.1M-potassium phosphate buffer, pH 7.6 (1.2 ml.), 0.1M-nicotinamide (0.3 ml.), 0.1 mM-cytochrome c (0.2 ml.) and 1.0M-sorbitol (0.1 ml.) (replaced by water in controls). Centre wells contained 0.1 ml. of 5N-NaOH. The gas phase was air. Incubation was for 1 hr. at an average temperature of 38° . Expts. 1-4 and 1a-4a were performed on different birds. In Expts. 1-3 the birds were pullets aged 5-10 weeks, and in Expts. 1-2 they were starved for 24 hr. In Expt. 4 a laying bird aged 26 weeks was used. Expts. 1a and 2a were performed with cockerels aged 14 weeks, and Expts. 3a and 4a with pullets aged 14 weeks.

Expt. no.	Substrate	$-Q_{O_2}$			Expt. no.	O_2 uptake (μ l.)	Extra O_2 uptake (μ l.)
		0-60 min.	60-120 min.	120-180 min.			
1	Fructose	17.3	17.4	16.6	1a	—	—
	Sorbitol	8.4	4.2	4.0		193	154
	Nil	8.0	4.0	2.6		39	—
2	Fructose	18.5	13.5	13.5	2a	—	—
	Sorbitol	14.5	9.0	8.5		130	111
	Nil	13.0	9.0	8.0		19	—
3	Fructose	12.8	12.0	12.2	3a	—	—
	Sorbitol	12.4	7.2	6.0		199	167
	Nil	10.8	8.6	8.0		32	—
4	Fructose	15.5	15.5	15.5	4a	—	—
	Sorbitol	9.0	6.5	6.5		252	217
	Nil	9.0	9.0	5.5		35	—

Table 2. *Effect of temperature of incubation on the fructose consumption and oxygen uptake of slices of rat and fowl liver*

Tissues were cut and incubated for 3 hr. in the presence of 10 mM-fructose as described in the Materials and Methods section. The gas phase was oxygen. Fructose was estimated with resorcinol (Roe, 1934). In Expt. 1 the bird and rat were fed before being killed. In Expt. 2 they were starved for 24 hr. $-q_{\text{fructose}}$ is defined as mg. of fructose consumed/mg. dry wt. of tissue/hr.

Expt. no.	Temperature	Tissue	$-q_{\text{fructose}}$	$-Q_{O_2}$	
				With fructose	Without fructose
1	41.3°	Chicken liver	0.0729	22.1	18.0
		Rat liver	0.0752	16.0	15.8
	37.5	Chicken liver	0.0637	16.5	15.3
		Rat liver	0.0672	14.0	15.0
2	41.3	Chicken liver	0.0626	11.8	4.7
		Rat liver	0.0289	6.4	5.9
	37.5	Chicken liver	0.0727	6.9	4.0
		Rat liver	0.0580	7.3	6.2

and the decline in the rate of oxygen uptake was more rapid than at 37.5°. When both bird and rat had been fed, fructose consumption was the same with both tissues even though the oxygen uptake was markedly different.

Independence of glucose and fructose metabolism. In mammalian liver the metabolism of fructose proceeds by a route independent of that of glucose (Hers, 1957), and is not markedly inhibited by glucosamine. With slices of fowl liver, glucosamine, at concentrations which strongly inhibited respiration in the presence of glucose (Fig. 2) and reduced

glucose uptake by over 80 %, decreased the oxygen uptake with fructose as a substrate to a much smaller extent and similarly did not strongly inhibit fructose consumption. Glucose at concentrations of 100 mM was without effect either on respiration in the presence of fructose or on fructose consumption (Table 3).

Gas exchange in a bicarbonate-buffered medium. Under aerobic conditions in a bicarbonate-buffered medium, the 'composite curve' representing the summation of carbon dioxide output and oxygen uptake was consistently positive though variable in magnitude when fructose was a substrate, and generally negative in the presence of glucose or in the absence of added substrate (Fig. 3). The extent to which the gas exchange with fructose as substrate was positive differed from bird to bird, as did the extent to which the curves with glucose as substrate were negative. Nevertheless, the general pattern of Fig. 3 was found with young pullets of 4-10 weeks, mature laying birds, birds in moult

Fig. 2. Effect of glucosamine on the oxygen uptake of slices of fowl liver metabolizing fructose and glucose. The bird, aged 10 weeks, was starved for 18 hr. Slices were incubated in phosphate-buffered saline at 41.3°. The gas phase was oxygen. ○, With fructose (10 mM); ●, with fructose (10 mM) and glucosamine (50 mM); ■, with glucose (10 mM) or no substrate; ▲, with glucose (10 mM) and no substrate and glucosamine (50 mM).

Fig. 3. Gas exchange of slices of fowl liver in a bicarbonate-buffered medium. The bird, aged 26 weeks, was in lay. Slices were incubated in bicarbonate-buffered saline at 41.3°. The gas phase was O₂ + CO₂ (95:5). ○, With fructose (10 mM); ●, with glucose or no substrate.

Table 3. *Effect of glucose and glucosamine on the fructose consumption of liver slices from the domestic fowl*

Birds, aged 7-8 weeks, were fed up to the time of death except in Expt. 2, where the bird was starved for 18 hr. Slices of liver were cut and incubated as described in the Materials and Methods section. Incubation was from 2.5 to 3.0 hr. at 41.3° under the appropriate gas phase. Expts. 1-3 were performed in a phosphate-buffered medium, and Expt. 4 with a bicarbonate-buffered medium. Glucose and fructose, when added as substrates, were included in the medium at a final concentration of 10 mM.

Expt. no.	Addition	Final concn. (mM)	-q _{fructose}		-q _{fructose} with addition as % of value without addition	-q _{glucose}		Rate with glucosamine as % of rate without glucosamine
			Without addition	With addition		Without glucosamine	With glucosamine	
1	Glucosamine	100	0.0690	0.055	80.0	0.00858	0.00151	17.7
2	Glucosamine	50	0.020	0.0121	60.0	0.0238	0.0039	16.4
3	Glucose	100	0.0648	0.0626	96.5	—	—	—
4	Glucose	100	0.0535	0.0495	92.5	—	—	—

and in the mature and immature cockerel. Chemical estimation of lactic acid produced during incubation showed that the positive production of carbon dioxide in the presence of fructose was not due to an excessive production of lactic acid. Pyruvic acid could not be detected. The results of several determinations are presented in Table 4. Under similar conditions with rat liver the total carbon dioxide output balanced reasonably well with the total lactic acid produced.

Respiratory quotient of avian liver. A production of excess of carbon dioxide during the metabolism of substrate has been previously studied with mam-

mary tissue from the lactating rat or guinea pig (see Folley & McNaught, 1961) and with adipose tissues (Ball & Cooper, 1960), and has been shown to be associated with R.Q. in excess of 1.0 and the synthesis of lipid or fatty acids. When measured in a medium buffered with bicarbonate the R.Q. of slices of liver from the domestic fowl was found to be 1.1 when fructose was the substrate and less than 1.0 in the presence of glucose or in absence of added substrate (Table 5). Under similar conditions slices of liver from the guinea pig yield R.Q. values of 0.70 with fructose and of 0.59 without fructose (cf. Dickens & Simer, 1931). If the birds were

Table 4. *Lactate and carbon dioxide production by slices of avian and mammalian liver metabolizing fructose*

Slices were cut and incubated at 41.3° for 3 hr. in bicarbonate-buffered saline as described in the Materials and Methods section. Values are expressed as μ moles/10 mg. dry wt. of tissue and are corrected for zero-time controls.

Expt. no.	Source of tissue	Substrate	Lactate production		Gas exchange (CO ₂)		CO ₂ in excess of lactate
			Total	In excess of control without substrate	Total	In excess of control without substrate	
1	Non-laying hen aged 4.5 weeks	Fructose	0.55	0.33	+0.55	+1.79	+1.46
		Glucose	0.32	0.09	-1.83	+0.09	0.0
		Nil	0.22	—	-1.74	—	—
2	Non-laying hen aged 5.0 weeks	Fructose	0.42	0.14	+2.42	+5.20	+5.06
		Glucose	0.52	0.24	-0.92	+1.84	+1.60
		Nil	0.28	—	-2.78	—	—
3	Laying hen aged 25.0 weeks	Fructose	0.50	0.25	+5.62	+4.36	+4.11
		Glucose	0.45	0.20	+3.34	+2.08	+1.80
		Nil	0.24	—	+1.26	—	—
4	Rat (adult)	Fructose	1.72	-0.37	+2.62	+0.06	+0.43
		Glucose	3.18	+0.99	+2.31	+0.25	-0.74
		Nil	2.19	—	+2.56	—	—

Table 5. *Respiratory quotient of slices of liver from the domestic fowl in the presence of different substrates*

Slices of liver were cut and incubated in a bicarbonate-buffered medium at 41.3° for 3 hr. as described in the Materials and Methods section. The R.Q., Q_{O_2} and Q_{acid} were determined as described by Dickens & Simer (1931). Substrates were added to a final concentration of 10 mM. Values are quoted $\pm$ s.e.m.

Age of birds (weeks)	No. examined	Physiological state	Substrate	R.Q.		$-Q_{O_2}$	Q_{acid}
				Mean	Range		
4.5-13.5	18	Non-laying Fed	Fructose	1.13 $\pm$ 0.04	0.81-1.36	+12.8 $\pm$ 0.66	+2.39 $\pm$ 0.30
			Glucose	0.94 $\pm$ 0.01	0.85-1.15	+11.23 $\pm$ 0.66	+2.02 $\pm$ 0.13
			Nil	0.86 $\pm$ 0.03	0.73-1.04	+11.08 $\pm$ 1.8	+2.0 $\pm$ 0.23
20-55	13	In lay Fed	Fructose	1.10 $\pm$ 0.02	0.96-1.26	+10.0 $\pm$ 0.65	+2.21 $\pm$ 0.19
			Glucose	0.85 $\pm$ 0.12	0.76-1.03	+9.4 $\pm$ 0.55	+2.1 $\pm$ 0.2
			Nil	0.77 $\pm$ 0.10	0.63-0.97	+9.5 $\pm$ 8.6	+1.98 $\pm$ 0.2
4.5-6.0	3*	Non-laying Starved for 24 hr.	Fructose	0.97	0.93-1.06	14.3	+1.27
			Glucose	0.83	0.78-0.90	13.7	+1.65
			Nil	0.71	0.53-0.85	9.4	+1.76
54	1	In lay Starved for 48 hr.	Fructose	0.87	—	7.5	+1.7
			Glucose	0.68	—	8.9	+2.6
			Nil	0.69	—	9.1	+2.6
16-30	3*	Cockerel Fed	Fructose	1.05	0.91-1.15	-11.1	+1.63
			Glucose	0.85	0.61-0.95	-11.1	+1.0
			Nil	0.73	0.63-0.79	-9.3	+0.96

* Values in these experiments are the average of the three determinations.

starved for 24–48 hr., fructose, though increasing the R.Q., did not raise it to the levels obtained if the birds were fed up to the moment of death. A marked sex difference could not be detected. Thus with the cockerel the R.Q. in the presence of fructose still remained slightly in excess of 1.0, whereas with glucose, or in the absence of added substrate, the values were similar to those obtained with laying and non-laying hens.

In both groups of pullets, whether fed or starved, the value of Q_{acid} remained close to 2.0. The nature of the acid produced is not known. Chemical determination of lactic acid in the flasks at the end

Fig. 4. Respiratory quotient of slices of fowl liver in a bicarbonate-buffered medium at different times during incubation. The birds were aged 8–10 weeks. Slices of liver were incubated with and without fructose in bicarbonate-buffered saline at 41.3°. The R.Q. was determined by the method of Dickens & Simer (1931). ○, With fructose (10 mM); ●, without fructose. Values are means $\pm$ s.e. of at least three determinations.

Table 6. *Respiratory quotient and Q_{O_2} of slices of pigeon liver in a bicarbonate-buffered medium*

Slices of liver from fed birds were cut and incubated at 41.3° for 3 hr. in a bicarbonate-buffered medium as described in the Materials and Methods section. Substrates were present at a final concentration of 10 mM. Birds in Expts. 1–3 were in lay with mature follicles in the ovary; bird no. 3 had a soft-shelled egg in the uterus. Bird no. 4 was not in lay; the ovarian follicles were not developed. Each value is the average of duplicate determinations.

Expt. no.	Substrate	R.Q.	$-Q_{O_2}$	Q_{acid}
1	Fructose	0.85	13.9	+1.85
	Glucose	0.82	11.8	+1.90
	Nil	0.55	8.7	+3.2
2	Fructose	0.82	9.4	+3.7
	Glucose	0.94	11.7	+1.5
	Nil	0.86	10.1	+2.2
3	Fructose	0.86	12.3	+2.6
	Glucose	0.74	10.2	+2.9
	Nil	0.64	10.3	+3.1
4	Fructose	0.94	11.3	+1.0
	Glucose	0.87	9.4	+0.7
	Nil	0.77	10.9	+1.6

of several experiments showed that only 5–25 % of the Q_{acid} value could be accounted for as lactic acid. Also, in contrast with respiration in a phosphate-buffered medium, the $-Q_{O_2}$ was not markedly different in the presence or absence of fructose provided that the birds had been fed.

Measurement of the R.Q. was normally made after an incubation period of 3 hr., this time being chosen as sufficient to yield a gas exchange large enough to allow accurate measurement (cf. Dickens & Simer, 1931). However, although the oxygen uptake in the presence of fructose was linear when measured in a phosphate-buffered medium over this period, there was no indication that this was so in a medium buffered with bicarbonate. Since the R.Q. is calculated from a single measurement after incubation for 3 hr., variation in the rate of oxygen uptake over this period could result in erroneous values (cf. Laser, 1942). The R.Q. was therefore measured at intervals over 0.5–3.0 hr. Fig. 4 shows that the R.Q. in the presence of fructose was essentially constant over a period of 3 hr. The larger scatter of results at 0.5 hr. is probably due to the difficulty of accurate measurement at this point. The $-Q_{O_2}$ values in these experiments were: at 0.5 hr., 10.0 ± 1.4 (3); at 1.0 hr., 10.7 ± 0.7 (4); at 2.0 hr., 11.6 ± 0.1 (3); at 3 hr., 11.6 ± 0.9 (5) (the numbers in parentheses refer to the numbers of experiments). In the absence of fructose the R.Q. steadily declined.

Respiratory quotient with pigeon liver. It was of interest to see whether another avian species also showed both an increased respiration and a R.Q. in excess of 1.0 when metabolizing fructose. With slices of pigeon liver fructose increased the oxygen uptake, analogous to its effect on liver from the domestic fowl (cf. Fig. 1). However, the R.Q. was not increased above that in the presence of glucose and remained less than 1.0 (Table 6).

DISCUSSION

The results described above clearly show that the metabolism of carbohydrate by slices of liver from the domestic fowl is quantitatively different from that of mammalian liver in several ways.

First, fructose is capable of maintaining an increased respiration in a phosphate-buffered medium. In mammalian liver, such as that from the rat, fructose does not usually enhance oxygen uptake (Dickens & Greville, 1933; Kratzing, 1961; see also Table 1), though the results obtained by Haydon (1961) suggest that this may sometimes occur. Of a large number of tissues examined the respiration of only nervous tissue and kidney was increased by fructose (Dickens & Greville, 1933).

Secondly, fructose maintained a R.Q. of 1.10 in

liver slices from both laying and non-laying hens. This observation does not appear to have been recorded with any mammalian tissue (cf. Kratzing, 1961). Thus, of rat tissues, the oxygen uptake of which is increased by fructose, kidney slices and cerebral tissues metabolized this substrate with R.Q. values of 0.90 and 1.0 respectively. With rat liver the R.Q. was 0.76; this value compares with the value of 0.70 obtained with guinea-pig liver in the present work. In lactating mammary gland from the rat a R.Q. in excess of 1.0 was obtained only with glucose (Folley & French, 1949), fructose neither supporting respiration nor increasing the R.Q. A similar result has been reported with rat epididymal fat pads (Pozza & Ghidoni, 1962).

The increased R.Q. was not found with slices of liver from birds starved for 18–24 hr. In the fowl (Weber, Banerjee, Bixler & Ashmore, 1961), as in the rat (Weber & McDonald, 1961), starvation decreases the amounts of enzymes that are involved in glucose catabolism though a starvation period of 6 days was required for the effect to be measurable. However, metabolic defects are apparent before a measurable depletion of enzymes is obtained. Thus, with rat liver, lipogenesis from fructose or glucose was decreased by starvation for 24 hr. (Wyshak & Chaikoff, 1953). Presumably a similar state was obtained with starved birds in the present experiments.

Of other carbohydrates tested only sorbose increased the Q_{O_2} in a phosphate-buffered medium. Even so, in a bicarbonate-buffered medium the R.Q. remained at 0.95 ± 0.05 (4 experiments), or 84 % of the R.Q. of other slices of the same liver metabolizing fructose in the same experiment.

The consumption of fructose by the liver of the domestic fowl was not inhibited by a 12-fold concentration of glucose, and neither was the oxygen uptake (Table 3 and Fig. 3). Similarly glucosamine, at concentrations strongly inhibiting both oxygen uptake and glucose consumption, had relatively little effect on oxygen uptake in the presence of fructose or on fructose consumption.

A simple interpretation of these results could be that the hexokinase of fowl liver possesses a markedly greater affinity for fructose than for glucose or for glucosamine. Though information for this tissue is lacking, in all mammalian tissues so far examined, and in yeast, the K_m of the hexokinases with glucose as a substrate is about one-hundredth that with fructose as a substrate (Crane, 1961). Further, the phosphorylation of fructose by preparations of tissue hexokinase is strongly inhibited by glucose (Crane & Sols, 1955). Hexose metabolism via hexokinase is also inhibited by glucosamine (Grant & Long, 1952; Brown, 1951). It is considered unlikely that the hexokinase of avian liver will prove markedly different in these respects.

It may be of significance that the $-q_{\text{glucose}}$ of avian liver (Table 3) is similar in magnitude to the value of 0.016 found by Long (1952) as the maximal rate of phosphorylation of glucose by fortified dispersions of rat liver.

An alternative explanation is the presence of a fructokinase similar in specificity to that of mammalian liver (Hers, 1957). In this system fructose is phosphorylated to form fructose 1-phosphate, which is split via 1-phosphofructoaldolase to yield dihydroxyacetone phosphate and D-glyceraldehyde. D-Sorbose yields dihydroxyacetone phosphate and L-glyceraldehyde. Metabolism of dihydroxyacetone may then proceed (a) by isomerization to glyceraldehyde 3-phosphate and metabolism via the Embden-Meyerhof pathway, (b) by condensation with glyceraldehyde 3-phosphate and thence to form glycogen (Leuthardt, Testa & Wolf, 1953), or (c) by dehydrogenation to L- α -glycerophosphate. D-Glyceraldehyde, in addition to the well-known route of reduction to glycerol and phosphorylation to L- α -glycerophosphate (see Hers, 1957, for references), is also oxidized directly to glyceric acid and then phosphorylated to 2-phosphoglycerate (Ichihara & Greenberg, 1957; Lamprecht & Heinz, 1958; Lamprecht, Diamantstein, Heinz & Balde, 1959) thus bypassing most of the Embden-Meyerhof pathway.

If similar routes exist in avian liver a simple explanation for the increased oxygen uptake in the presence of fructose could lie in the rate at which fructose is converted into pyruvate for subsequent oxidation via the tricarboxylic acid cycle. This in turn must involve a quantitative difference in metabolism at some point for in mammalian liver fructose is largely converted into glucose or glycogen (Hers, 1957), up to 80 % appearing as glucose in slices of rat liver (Beloff-Chain, Catanzaro, Chain, Masi & Pocchiari, 1955). Nevertheless, with slices of both rat and fowl liver under similar conditions the consumption of fructose was almost identical (Table 2, Expt. 1).

The ability of fowl-liver systems to synthesize fatty acids, triglycerides and phospholipids is well documented (for reviews see Kennedy, 1957; Stumpf, 1960; Langdon & Phillips, 1961). In such a tissue a rapid fructose metabolism primarily channelled through a route both leading to the formation of glycerophosphate and avoiding the Embden-Meyerhof pathway could therefore be conceived as leading to enhanced lipogenesis and a R.Q. in excess of 1.0. Nevertheless, there are differences in avian species, for slices of pigeon liver, though metabolizing fructose with an increased oxygen uptake, did so with a R.Q. less than 1.0. This in turn may be related to the greater capacity for egg production in the domestic fowl, with an accompanying increased ability to synthesize lipid

in the liver (Ranney, Chaikoff & Entenman, 1951; Vanstone, Dale, Oliver & Common, 1957) but further work is needed to clarify both this and the other points raised above.

SUMMARY

1. Slices of liver from the domestic fowl metabolize fructose with an increased oxygen uptake, in contrast with mammalian liver where fructose metabolism does not increase the oxygen uptake.

2. Fructose metabolism by fowl liver was not affected by glucose added in a tenfold excess and was only slightly inhibited by glucosamine at concentrations almost completely suppressing glucose metabolism.

3. In a bicarbonate-buffered medium the metabolism of fructose was accompanied by a steady gas output not wholly accounted for by the formation of lactic acid.

4. Determination of the respiratory quotient, R.Q., showed that slices of fowl liver from fed birds metabolized fructose with a R.Q. of 1.10. This occurred in both laying and non-laying pullets. Liver from the cockerel yielded essentially similar results.

5. Slices of pigeon liver, though showing an increased oxygen uptake with fructose, did not metabolize the sugar with a R.Q. greater than 1.0.

6. Fowl liver has been shown to possess sorbitol-dehydrogenase activity though slices do not show an increased respiration in the presence of sorbitol.

7. The results are discussed in relation to known pathways of fructose metabolism in mammalian liver.

The expert assistance of Miss Patricia M. Baxendale is gratefully acknowledged.

REFERENCES

- Ball, E. G. & Cooper, O. (1960). *J. biol. Chem.* **235**, 584.
 Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
 Beloff-Chain, A., Catanzaro, R., Chain, E. B., Masi, I. & Pocchiari, S. (1955). *Ann. Acad. Sci. fenn. (ser. A, sect. 2)*, **60**, 412.
 Blakley, R. L. (1951). *Biochem. J.* **49**, 257.
 Brown, D. H. (1951). *Biochim. biophys. Acta*, **7**, 487.
 Crane, R. K. (1961). In *Biochemists' Handbook*, p. 401. Ed. by Long, C. London: E. and F. N. Spon Ltd.
 Crane, R. K. & Sols, A. (1955). In *Methods in Enzymology*, vol. 1, p. 277. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Dickens, F. & Greville, G. D. (1933). *Biochem. J.* **27**, 832.
 Dickens, F. & Simer, F. (1931). *Biochem. J.* **25**, 62.
 Folley, S. J. & French, T. H. (1949). *Biochem. J.* **45**, 117.
 Folley, S. J. & McNaught, M. L. (1961). In *Milk: The Mammary Gland and its Secretion*, vol. 1, p. 441. Ed. by Kon, S. K. & Cowie, A. T. London: Academic Press Ltd.
 Grant, P. T. & Long, C. (1952). *Biochem. J.* **51**, xx.
 Hassid, W. Z. & Abraham, S. (1957). In *Methods in Enzymology*, vol. 3, p. 34. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
 Haydon, R. K. (1961). *Biochim. biophys. Acta*, **46**, 598.
 Hers, H. G. (1957). *Le Métabolisme du Fructose*. Brussels: Éditions Arscia.
 Ichihara, A. & Greenberg, D. M. (1957). *J. biol. Chem.* **225**, 949.
 Keilin, D. & Hartree, E. F. (1948). *Biochem. J.* **42**, 221.
 Kennedy, E. P. (1957). *Annu. Rev. Biochem.* **26**, 119.
 Kratzing, C. C. (1961). In *Biochemists' Handbook*, p. 795. Ed. by Long, C. London: E. and F. N. Spon Ltd.
 Krebs, H. A. & Henseleit, K. (1932). *Hoppe-Seyl. Z.* **210**, 33.
 Lamprecht, W., Diamantstein, T., Heinz, F. & Balde, P. (1959). *Hoppe-Seyl. Z.* **316**, 97.
 Lamprecht, W. & Heinz, F. (1958). *Z. Naturf.* **136**, 464.
 Langdon, R. G. & Phillips, A. H. (1961). *Annu. Rev. Biochem.* **30**, 189.
 Laser, H. (1942). *Biochem. J.* **36**, 319.
 LePage, G. A. (1957). In *Manometric Techniques*, p. 268. Ed. by Umbreit, W. W., Burris, R. H. & Stauffer, J. F. Minneapolis: Burgess Publishing Co.
 Leuthardt, F., Testa, E. & Wolf, H. P. (1953). *Helv. chim. acta*, **36**, 227.
 Long, C. (1952). *Biochem. J.* **50**, 407.
 McIlwain, H. (1951). *Biochem. J.* **49**, 383.
 McIndoe, W. M. (1959). *Biochem. J.* **72**, 153.
 Mann, T. (1946). *Biochem. J.* **40**, 481.
 Pozza, G. & Ghidoni, A. (1962). *Clin. chim. Acta*, **7**, 55.
 Ranney, R. E., Chaikoff, I. L. & Entenman, C. (1951). *Amer. J. Physiol.* **165**, 596.
 Roe, J. H. (1934). *J. biol. Chem.* **107**, 15.
 Stumpf, P. K. (1960). *Annu. Rev. Biochem.* **29**, 261.
 Sturkie, P. D. (1954). *Avian Physiology*. Ithaca, N.Y.: Comstock Publications Association.
 Vanstone, W. E., Dale, D. G., Oliver, W. F. & Common, R. H. (1957). *Canad. J. Biochem. Physiol.* **35**, 659.
 Weber, G., Banerjee, G., Bixler, D. & Ashmore, J. (1961). *J. Nutr.* **74**, 157.
 Weber, G. & McDonald, H. (1961). *Exp. Cell Res.* **22**, 292.
 Wyshak, G. H. & Chaikoff, I. L. (1953). *J. biol. Chem.* **200**, 851.