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Nicotinamide Nucleotide Coenzymes and Glucose Metabolism in the Livers of Foetal and Newborn Lambs

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Many physiological adjustments take place in the newly born animal. Some of these are associated with cardiovascular changes (Dawes, 1961; Mott, 1961), and others can be more closely related to metabolism. During the later part of foetal development, the glycogen content of the liver rises to high levels and falls rapidly immediately after birth in many animals (Shelley, 1960). Similar changes occur in the lipid content of the liver of the guinea pig in the perinatal period (Raiha, 1961). Dawkins (1959) has shown that respiratory enzymes in the liver of the newborn rat increased in activity in the first few days after birth. In the pre-natal guinea pig, activities of glucuronyltransferase, tryptophan pyrrolase and glucose 6-phosphatase are low, and rise toward adult values soon after birth, whereas that of glucose 6-phosphate dehydrogenase shows a gradual decrease and that of 6-phosphogluconate dehydrogenase shows little change during development (Nemeth & Dickerman, 1960). These changes in relative enzymic activities and in carbohydrate and lipid contents

reflect the changes in functional activities of the liver after birth (see Villee, 1961).

Caiger, Morton, Filsell & Jarrett (1962) have shown that during development of the rat and the sheep the concentration of total nicotinamide nucleotide coenzymes in the liver increased and reached a maximum value as growth ceased. The changes in concentrations of coenzymes and the relative stage of development of the animal were assessed by using a number of parameters including liver wet wt., liver dry wt. and number of nuclei/liver.

In the studies of these coenzymes (expressed as amount/g. fresh wt.) in the livers of foetal, newborn and adult guinea pigs (Nemeth & Dickerman, 1960; Raiha, 1961) and of rabbits (Roux, Gordon, Dinnerstein & Romney, 1962), similar trends in relation to age were observed.

The present paper describes relationships of nicotinamide nucleotide coenzymes to growth and metabolism, and includes measurements on livers of foetal and newborn lambs as well as on older

sheep. The results confirm and extend those reported by Caiger *et al.* (1962). The oxidative pentose phosphate pathway is a potential source of NADPH₂ for reductive synthesis (Dickens, 1961; Lowenstein, 1961), and relative activities of glucokinase, glucose 6-phosphate dehydrogenase and of 6-phosphogluconate dehydrogenase were measured.

EXPERIMENTAL

Animals

Sheep. Merino lambs (48 animals) ranging in age from about 2 weeks before birth to 50 days after birth, five wethers (6 months to 4 years old) and two adult ewes (5–6 years old) were used; the gestation period of merino sheep under local conditions is about 150 days.

The lambs listed in Table 1 were born during May from ewes that had been grazing on an improved mixed pasture, dry until the end of April, and becoming increasingly green after autumn rains. These ewes were also offered a supplementary feed of chaffed wheat hay, and during the last few weeks of pregnancy an additional ration of grain (oats and wheat). A further group of lambs (Table 2) born during August–September were also used. These animals grazed on an improved mixed pasture (spring growth) and the lambs ran with their dams in a separate area on the same type of pasture.

The prenatal lambs were removed from the uterus within 30 sec. of slaughtering the ewe, and placed on a weighed stainless-steel tray. The curved crown–rump length (Cloete, 1939) was measured after all samples had been obtained. The newborn lambs (1–5 hr. old) were unsuckled; the other lambs and sheep were removed from their source of food and water about 2 hr. and the rats about 30 min. before slaughter.

Rats. Albino rats, 6 months old (body wt. 470–510 g.), were from the same colony as that used by Caiger *et al.* (1962).

Assay procedures

Sampling procedure and determinations of nicotinamide nucleotide coenzymes, dry wt., nitrogen and nuclei/g. fresh wt. of tissue were carried out as described by Caiger *et al.* (1962).

Nucleic acids. These were extracted as described by Schneider (1945) except that 5% (w/v) perchloric acid was used to remove acid-soluble material and DNA was determined colorimetrically with *p*-nitrophenylhydrazine (Webb & Levy, 1955); the *N*-NaOH used to develop the colour was saturated with trisodium phosphate at 20°. The DNA contents of about one-third of the samples were also determined by Burton's (1956) procedure. The values obtained by Burton's (1956) method were up to 5% lower than those obtained by Webb & Levy's (1955) procedure. Phosphorus was determined by Allen's (1940) procedure. Calf-thymus DNA (Zamenhof, 1958) was used as a standard; as determined by Wyatt's (1955) procedure, the uracil: thymine molar ratio was no greater than 0.02:1 and the recovery of bases was 96% of the total phosphorus.

The tissue samples were treated with perchloric acid and lipid solvents, and the dried powder was stored at –15° for less than 5 days before extraction with trichloroacetic acid and determination of deoxyribose. Storage of the dried

powder for more than 1 week or storage of the trichloroacetic acid extract for more than 12 hr. gave lower values of DNA. Some samples of butyl acetate used in Webb & Levy's (1955) method contained an impurity that was not removed by distillation; this interfered with colour development. Satisfactory results were obtained with a sample from Judex Chemical Co. Ltd. (England).

Relative enzymic activities

Rates of reduction of NADP with glucose 6-phosphate, with 6-phosphogluconate and with glucose and ATP were measured in the presence of tissue extracts; the methods used were based on those of Glock & McLean (1953) and of McLean (1958).

Approx. 2 g. of tissue was weighed and transferred to 18 ml. of ice-cold 0.15M-KCl–0.16 mM-KHCO₃ in a Potter–Elvehjem glass mortar with a Teflon pestle (A. H. Thomas and Co., Baltimore, U.S.A.). The tissue was dispersed by hand for 30 sec. and then homogenized at 1000 rev./min. for 1 min. The homogenate was centrifuged at 60000g for 30 min. at 2° and the supernatant dialysed at 2° against the KCl–KHCO₃ solution for about 18 hr. The extract was again centrifuged at 28000g for 15 min. at 2°. Reduction of NADP was measured at 340 mμ in cuvettes of 1 cm. light-path at 25° with a Beckman DK–2A recording spectrophotometer. Each cuvette contained 0.5 ml. of 25 mM-glycylglycine–KOH buffer, pH 7.6, 0.5 ml. of 0.1M-MgCl₂, 0.2 ml. of 1.5 mM-NADP and liver extract (0.1 ml. for dehydrogenase activity and 0.2 ml. for glucokinase activity) in a total volume of 2.4 ml. The glucose 6-phosphate, 6-phosphogluconate and glucose respectively were added to the test cuvettes in each of the appropriate assays (see below).

Rate of reduction of nicotinamide–adenine dinucleotide phosphate with glucose 6-phosphate. The reaction was started by the addition of 0.1 ml. of 50 mM-glucose 6-phosphate. The rate of change of extinction at 340 mμ was linear for at least 8 min. The results are expressed as μmoles of NADPH₂ formed/min./g. fresh wt.

Rate of reduction of nicotinamide–adenine dinucleotide phosphate with 6-phosphogluconate. The reaction was started by the addition of 0.1 ml. of 0.1M-6-phosphogluconate. The rate of change of extinction at 340 mμ declined somewhat with time, particularly after the first 2 min. Results were expressed as μmoles of NADPH₂ formed/min./g. fresh wt., calculated from the rate of change of extinction in the first 2 min. The same rate was observed on addition of 0.1 ml. of 50 mM-6-phosphogluconate to the test cuvette.

Rate of reduction of nicotinamide–adenine dinucleotide phosphate with glucose and adenosine triphosphate. ATP (0.4 ml. of a 20 mM solution) was added to both test and blank cuvettes. The reaction was started by the addition of 0.1 ml. of 2.5M-glucose. After a lag period of up to 2 min. the rate of increase of extinction was constant for at least 16 min. The results were expressed as μmoles of NADPH₂ formed/min./g. fresh wt. as determined after the lag period. With lower concentrations of glucose, lower rates were observed; from the results an apparent Michaelis constant, *K_m*, for glucose was determined at 14 mM. All results shown in Fig. 2 (*a*) are given for a substrate concentration of 0.1M-glucose.

Chemicals. Glucose 6-phosphate (sodium salt) and 6-phosphogluconate (sodium salt) were obtained from C. F. Boehringer und Soehne (Germany) and Sigma Chemical Co.

Table 1. *Reference bases and concentrations of nicotinamide nucleotide coenzymes in livers of lambs and sheep*

Experimental details are given in the text.

Age	Body wt. (kg.)	Crown-rump length (cm.)	Liver fresh wt. (g.)	Liver dry wt. (g.)	Nitrogen (mg./g. fresh wt.)	$10^{-6} \times$ Nuclei/g. fresh wt.	Coenzyme content (μ m-moles/g. fresh wt.)								Total
							[NAD] [NADH ₂]	[NAD] [NADH ₂]	[NAD] [NADH ₂]	[NADP] [NADPH ₂]	[NADP] [NADPH ₂]	[NADP] [NADPH ₂]	[NADP] [NADPH ₂]	[NADP] [NADPH ₂]	
Prenatal	2.8	49.5	86.0	19.0	21.5	496	204	39	243	8	68	76	319	319	
	3.1	46.6	71.2	17.9	23.3	365	241	24	265	5	124	129	394	394	
	3.3	48.3	68.1	17.3	20.8	304	—	—	—	—	—	—	—	—	
	3.3	52.0	69.8	17.4	23.6	374	460	31	491	5	79	84	575	575	
	3.4	49.5	67.0	17.4	20.6	335	562	41	603	10	63	73	676	676	
	3.5	50.8	82.7	20.7	22.9	392	578	77	655	5	77	82	737	737	
	3.7	50.8	106	27.5	22.2	350	338	38	376	5	115	120	496	496	
	4.9	55.9	93.3	25.3	21.5	288	543	44	587	5	55	60	647	647	
	3.5	51.3	64.5	17.8	24.8	376	487	83	570	5	91	96	666	666	
	3.5	52.7	71.4	19.5	24.2	365	575	79	654	5	61	66	720	720	
Postnatal	3	51.4	60.6	15.3	28.0	369	407	65	472	5	117	122	594	594	
	3	55.2	70.2	18.3	27.1	347	480	—	—	5	—	—	—	—	
	2	56.6	71.1	18.8	26.7	412	584	60	644	5	120	125	769	769	
	2	55.9	95.6	26.5	27.2	329	763	44	807	5	184	189	996	996	
	(days)														
	1	53.4	93.0	22.3	30.5	291	566	66	632	10	92	102	734	734	
	1	55.9	79.5	21.6	30.5	314	408	90	498	15	139	154	652	652	
	1	59.4	97.1	24.3	27.5	316	578	57	635	5	81	86	721	721	
	1	59.7	129	32.3	31.6	296	321	75	396	5	98	103	499	499	
	2	64.6	97.5	25.2	29.0	297	314	44	358	5	65	70	428	428	
(years)	3	60.7	136	35.5	30.9	309	260	58	318	5	63	68	386	386	
	3	60.4	154	40.6	30.3	—	377	59	436	29	235	264	700	700	
	5	62.2	146	36.2	29.6	298	329	42	476	5	92	97	468	468	
	8	73.7	175	48.3	29.5	272	414	62	476	10	114	124	600	600	
	14	8.0	73.7	48.3	29.5	272	414	62	476	10	114	124	600	600	
	18	8.8	181	51.3	32.5	254	448	56	504	5	135	140	644	644	
	27	9.3	212	53.5	30.7	305	460	54	514	10	91	101	615	615	
	24	10.0	76.2	60.5	30.4	256	472	73	545	5	184	189	734	734	
	22	11.3	77.5	64.6	29.5	264	352	69	421	20	166	186	607	607	
	50	15.3	85.4	80.0	32.6	284	474	59	533	15	178	193	726	726	
(years)	5	—	493	145	31.6	275	585	58	643	15	197	212	855	855	
	6*	—	720	220	30.7	258	612	79	691	15	256	271	962	962	

* Lactating ewe, 24 days.

(U.S.A.) respectively. Glucose (AnalaR grade) was obtained from British Drug Houses Ltd., ATP (sodium salt) was from either C. F. Boehringer und Soehne (Germany) or from California Corp. for Biochemical Research (U.S.A.).

RESULTS

Reference bases. So that changes in amounts of coenzymes and in enzyme activities could be adequately assessed in a tissue that is rapidly developing, a number of parameters other than calendar age were determined. Tables 1 and 2 show the values for age, body wt., liver fresh wt., liver dry wt. and the number of nuclei and the amount of nitrogen/g. fresh wt. of liver. The crown-rump length was measured in prenatal lambs but it varied too much in relation to other parameters to

be a reliable guide to the stage of development. Table 2 includes values for nucleic acid phosphorus.

The percentage dry wt. of the livers in the animals up to 5 days old varied between 22.1 and 27.7 %, with a mean of 25.9 % (28 animals), and in the five adult sheep increased to a mean of 29.9 % (range 29.3–30.7 %). The total nitrogen/g. fresh wt. of liver was lower in the eight prenatal animals (mean 22.1, range 20.6–23.6 mg./g.) than in the six newborn (mean 26.3, range 24.2–28.0 mg./g.) and than in the older lambs and adults (41 animals) (mean 31.3, range 27.5–36.4 mg./g.).

Concentration of coenzymes. Table 1 shows the concentrations of nicotinamide nucleotide coenzymes in livers of prenatal and postnatal lambs of various ages, together with the corresponding values for the other parameters measured. A wide varia-

Table 2. *Reference bases and the concentration of nucleic acid phosphorus and of nicotinamide-adenine dinucleotide in livers of sheep and rats*

Experimental details are given in the text.

Animals	Age (days)	Body wt. (kg.)	Liver fresh wt. (g.)	Liver dry wt. (g.)	Nitrogen (mg./g. fresh wt.)	10 ⁻⁶ × Nuclei/g. fresh wt.	Nucleic acid phosphorus (μg./g. fresh wt.)		[NAD] (μm-moles/ g. fresh wt.)
							DNA	RNA	
Lambs	4	4.0	88.6	22.5	29.9	264	264	384	—
		4.0	84.5	23.0	29.4	269	347	517	432
		4.8	100	26.8	32.5	365	428	360	—
		5.3	108	29.3	29.6	380	354	416	—
		6.0	144	38.2	31.4	338	390	452	—
		6.7	161	42.4	29.5	307	316	476	370
	7	6.1	181	51.0	28.2	199	208	454	396
		6.5	154	40.6	31.5	262	—	—	310
		7.2	177	46.3	31.6	250	279	361	374
		8.0	218	58.2	30.4	233	272	541	374
	14	8.4	220	61.4	29.7	243	317	443	310
		8.5	218	57.9	29.6	250	325	381	277
		10.0	284	81.1	29.4	271	271	391	260
	15	8.0	199	54.5	29.8	249	302	398	370
	29	11.9	260	71.8	33.3	269	288	412	460
	30	12.5	232	62.8	33.1	312	378	346	475
	28	13.3	281	77.7	33.9	317	304	403	435
	26	15.6	341	94.9	29.6	289	317	473	420
	49	15.8	303	88.4	33.1	265	—	714*	504
	49 (months)	19.1	336	96.4	33.9	261	—	625*	584
Sheep	6	23.1	681	205	35.4	217	—	526*	636
	6 (years)	28.3	691	202	34.2	213	242	348	595
	3	56.6	921	275	33.8	188	192	318	717
	4	57.6	857	250	34.9	234	—	510*	824
	4 (months)	57.8	884	272	36.4	209	—	440*	910
	6	464	16.7	5.3	32.9	152	187	526	465
Rats		477	17.2	5.3	31.6	127	159	506	405
		494	17.2	5.5	32.4	143	162	508	415
		500	19.4	6.1	28.7	155	—	—	385
		510	19.8	6.2	27.4	150	—	480*	460

* Total nucleic acid phosphorus.

tion was observed in the values obtained for the livers of the foetal and newborn animals, but the general trend was for the concentration of total NAD/g. fresh wt. to be higher in the prenatal and immediately postnatal lambs than in lambs 2–5 days old. The concentration of total NADP/g. fresh wt. in the livers of the foetal and newborn animals was lower than in the young lambs and sheep. Values obtained for lambs during their subsequent development agreed well with those obtained by Caiger *et al.* (1962).

Table 2 shows the concentration of NAD in the livers of postnatal lambs and sheep and of adult rats.

Total amounts of coenzymes. From the results in Table 1 values for number of nuclei and amounts of coenzymes in the whole liver of each animal were derived. Regression analyses were carried out on those variates pertinent to this investigation and gave the following equations:

$$\begin{aligned} A, \log [\text{total coenzymes}] &= 0.2772 + 1.0606 \log W; \\ B, \log ([\text{NAD}] + [\text{NADH}_2]) &= 0.2613 + 1.0062 \log W; \\ C, \log ([\text{NADP}] + [\text{NADPH}_2]) &= -0.8834 + 1.3395 \log W; \\ D, \log [\text{total coenzymes}] &= -1.4834 + 1.2978 \log N; \\ E, \log ([\text{NAD}] + [\text{NADH}_2]) &= -1.3873 + 1.2228 \log N; \\ F, \log ([\text{NADP}] + [\text{NADPH}_2]) &= -3.2169 + 1.6814 \log N; \end{aligned}$$

where W is the liver dry wt. (g.) and N is the number of nuclei $\times 10^{-8}$. In each case the regression is highly significant ($P < 0.001$). A comparison was made of these six regressions and those obtained for growing lambs by Caiger *et al.* (1962; equations A–F in Fig. 1). Since only in one case, namely regression B, do the coefficients differ significantly and these only at the 0.05 level, the two sets of data may be considered to be compatible. The difference in regression B was due to higher

values of $([\text{NAD}] + [\text{NADH}_2])$ in the livers of the young lambs in the present series. The means of each of the regressions of the present series differ significantly from those of the previous series (Caiger *et al.* 1962) as would be expected from the differences in the stages of development of the animals in each group.

Coenzyme ratio. From the values of $([\text{NAD}] + [\text{NADH}_2])$ and of $([\text{NADP}] + [\text{NADPH}_2])$ obtained from the regression equations B and C, the coenzyme ratio

$$([\text{NAD}] + [\text{NADH}_2]) : ([\text{NADP}] + [\text{NADPH}_2])$$

was calculated for selected liver dry weights. Since the regression equations from the present and previous studies (Caiger *et al.* 1962) have been shown to be compatible the results of both series were pooled and gave the following equation:

$$y = 3.728 - 3.473x + 0.879x^2$$

where y is $\log \{([\text{NAD}] + [\text{NADH}_2]) : ([\text{NADP}]$

$+ [\text{NADPH}_2])\}$ and x is $\log [\text{liver dry wt. (g.)}]$.

The curve in Fig. 1 shows these relationships.

Enzymic activities. The rates of reduction of NADP in the presence of added glucose and ATP, glucose 6-phosphate and 6-phosphogluconate under the conditions used in the present work reflect the relative activities of glucokinase, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Figs. 2 (a), 2 (b) and 2 (c) show these activities for lambs and sheep (as μmoles of NADPH_2 produced/min./g. fresh wt.) plotted against liver dry wt. Logarithms were taken of both the dependent and determining variates to produce homoscedasticity and linearity, and regression analysis gave the curves shown.

The rate of reduction of NADP with 6-phosphogluconate was greater than that with glucose 6-phosphate. The relative activity with 6-phosphogluconate for prenatal and newborn lambs was 3.5–7.0 μmoles of NADPH_2 /min./g. fresh wt. and declined during further development to 0.4–2.5 μmoles of NADPH_2 /min./g. fresh wt. The relative activity with glucose 6-phosphate also showed a wide scatter, falling from about 1.0 to about 0.4 μmole of NADPH_2 /min./g. fresh wt. in the liver of the mature animal. With glucose and ATP the values were 0.3–1.0 μmole of NADPH_2 /min./g. fresh wt. in the prenatal and newborn lambs and fell to 0.01–0.05 in mature wethers.

Some of the wide variation in relative enzymic activities in the very young lambs (Fig. 2) could well have been due to differences in the previous nutritional history of lambs taken at random from the flock.

With extracts of rat liver, for rats listed in order of increasing body wt. (see Table 2), the following

Fig. 1. Change in coenzyme ratio

$$([\text{NAD}] + [\text{NADH}_2]) : ([\text{NADP}] + [\text{NADPH}_2])$$

in the liver during development of the sheep. The curve was derived from the equation given in the text.

values were obtained: with glucose 6-phosphate, 6.25, 5.78, 5.58, 1.55 and 2.55; with 6-phosphogluconate, 1.33, 1.39, 1.32, 1.21 and 1.26; with glucose and ATP, 0.36, 0.33, 0.34, 0.14 and 0.32 μmoles of $\text{NADPH}_2/\text{min./g.}$ fresh wt. respectively.

Nucleic acids. Values of DNA phosphorus and RNA phosphorus/g. fresh wt. of liver were deter-

mined in lambs and sheep from 4 days to 4 years old and in adult rats. The results are given in Table 2. Some samples deteriorated on storage (see the Experimental section) and, for these, values of total nucleic acid phosphorus only are shown in Table 2. RNA phosphorus was determined by difference between total nucleic acid phosphorus and DNA phosphorus. From these, the RNA phos-

Fig. 2. Change in the rate of reduction of NADP by homogenates of sheep and lamb liver during development: (a) with glucose and ATP; (b) with glucose 6-phosphate; (c) with 6-phosphogluconate.

Curves were drawn from the regression equations:

$$(a) \log y = 1.3182 - 1.2020 \log x; \quad (b) \log y = 0.4455 - 0.3459 \log x; \quad (c) \log y = 0.9705 - 0.4025 \log x;$$

where y is μmoles of $\text{NADPH}_2/\text{min./g.}$ fresh wt. and x is the liver dry wt. (g.). In (a) and (b) $P < 0.001$, in (c) $P < 0.05$. $\blacktriangle$, Values for lactating ewe.

phorus : DNA phosphorus ratio and the value of DNA phosphorus/nucleus were calculated. The mean value for the RNA phosphorus : DNA/phosphorus ratio was 1.40 (range 0.84–2.18) for 19 animals (sheep and lambs). The ratio showed no trend during development. This value was about half of that obtained for three adult rats (mean 3.04, range 2.81–3.18).

The DNA phosphorus/nucleus was found to be 1.12 $\mu\text{g.}$ (range 0.93–1.30 $\mu\text{g.}$) in these lambs and sheep, and 1.20 $\mu\text{g.}$ (range 1.13–1.25 $\mu\text{g.}$) in the adult rats. In a further group of pen-fed ewes aged 3 years, a mean value of 1.03 $\mu\text{g.}$ (range 0.91–1.19 $\mu\text{g.}$) for seven animals was obtained.

DISCUSSION

Reference bases in liver tissue. Caiger *et al.* (1962) discussed the importance of the choice of reference base when assessing changes in a rapidly developing tissue; in the present work the dry wt. of the liver and the total number of nuclei/liver have again been used as parameters of relative stages of development. In addition, nucleic acid phosphorus was determined for most of the animals listed in Table 2.

Little information is available on the DNA content of lamb and sheep liver. Values of 5.4 and 6.1 $\mu\text{g.}$ of DNA/nucleus for livers of two sheep were reported by Vendrely & Vendrely (1949); these results were based on determinations with Dische's (1930) diphenylamine reaction with the nucleic acid extract obtained by modification of Schneider's (1945) and Schmidt & Thannhauser's (1945) procedures. The mean DNA content of the nuclei in the livers of sheep and lambs used in the present study (19 animals) was 1.12 $\mu\text{g.}$ of DNA phosphorus/nucleus (equivalent to 11.2 $\mu\text{g.}$ of DNA/nucleus) and is thus much higher than the values obtained by Vendrely & Vendrely (1949).

The mean value of DNA phosphorus/nucleus in the livers of 6-month-old rats (1.12 $\mu\text{g.}$; see Table 2) was in good agreement with results of other workers (0.91–1.14 $\mu\text{g.}$ of DNA phosphorus/nucleus; see Leslie, 1955).

Whereas there was no trend in the amount of DNA phosphorus/nucleus during development in the sheep, the mean value of 1.2 $\mu\text{g.}$ for livers of rats at 6 months was higher than the value (0.77 $\mu\text{g.}$; Caiger *et al.* 1962) at 1 month, owing to the increased proportion of tetraploid nuclei in the older rats. The expression of coenzyme or enzyme concentrations as functions of either DNA phosphorus in the liver or of number of nuclei in the liver of sheep is equally valid, but in corresponding studies in rats allowances would have to be made for some increase in the average amount of DNA of the nuclei in the liver during development.

Changes in nicotinamide nucleotide coenzymes during growth. The present study included animals from about 2 weeks before birth to maturity.

The regression equations *A–F* (see above) that relate coenzyme content to stage of liver development in the sheep confirm the findings of Caiger *et al.* (1962), which were derived from studies with animals from 4 days old to maturity.

The small difference ($P < 0.05$) in the regression equation *B*, and the corresponding equation derived by Caiger *et al.* (1962, Fig. 1), is due to a higher concentration of NAD in the livers of animals during the first 2 weeks after birth. In the present study the growth rate of the young lambs was less than in the work by Caiger *et al.* (1962), probably reflecting a poorer nutritional status of the lambs, which were running with older dams on autumn pasture. Caiger *et al.* (1962) have shown that the concentration of nicotinamide nucleotide coenzymes is lower in a more rapidly growing liver, a finding consistent with the general relationship between rate of cell division and concentration of nicotinamide nucleotide coenzymes proposed by Morton (1958, 1961). The present study with a larger number of young animals also confirms the relationship between the rate of change of number of nuclei as a function of the rate of change of concentration of total coenzymes in the liver (see Caiger *et al.* 1962, Fig. 3).

Intermediary metabolism in the livers of the foetal and newborn lambs. A number of studies on the development of enzyme systems in the maturing mammalian liver have indicated a changing pattern to enable the liver to perform its important role of metabolic homeostasis after birth. In the later stage of foetal development liver metabolism is characterized by an increasing rate of synthesis and deposition of glycogen, the concentration of which falls rapidly in the first few hours after birth and, after remaining low for several days, gradually rises towards the adult level (Villev, 1954; Shelley, 1960). The ability of the liver to regulate the blood glucose concentration is linked with the development of glucose 6-phosphatase activity, which appears during the latter part of gestation and increases rapidly immediately after birth in all species that have been examined, e.g. rat, rabbit, guinea pig, sheep, man (see Dawkins, 1961).

Although the supply of exogenous glucose to the liver is limited in adult sheep it has been possible to show some glucokinase activity in liver homogenates from these animals. Gallagher & Buttery (1959) were unable to demonstrate glucokinase activity in liver homogenates from newborn lambs and adult sheep; these workers used 10 mM-glucose whereas the apparent K_m for glucose is 14 mM (see the Experimental section). With rat-liver slices Dipietro, Sharma & Weinhouse (1962) found that the re-

spiration with added glucose increased fivefold when the glucose concentration was increased from 10 to 100 mM; the apparent K_m for glucose was 20 mM. The high K_m value is consistent with the marked concentration-dependence of glucose utilization in the liver as observed by other workers (see DiPietro *et al.* 1962).

The values obtained with glucose and ATP reported in the present paper for the livers of foetal and newborn lambs (Fig. 2a) were of the same order as those found in adult rats (0.14–0.36 μ mole of NADPH₂/min./g. fresh wt.) and much higher than in adult sheep. Jarrett & Filsell (1958) have shown that the apparent hexokinase activity, as measured by the ability of intestinal mucosa to utilize glucose, is greater in 2-day-old lambs and adult rats than in older lambs and adult sheep.

Glucose 6-phosphate may be oxidized via the glycolytic and pentose phosphate pathways, both of which operate from an early stage of development in foetal liver (Villem & Loring, 1961). Figs. 2(b) and 2(c) show that the relative activities of the first two enzymes involved in the oxidative pentose phosphate pathway, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, also decrease during development of the liver in sheep. Nemeth & Dickerman (1960) have reported a similar fall in activity of glucose 6-phosphate dehydrogenase during development of guinea-pig liver, with little change in activity of 6-phosphogluconate dehydrogenase.

With adult rats the rates of reduction of NADP with 6-phosphogluconate were less than with glucose 6-phosphate, in contrast with the lambs and sheep. The values obtained with the liver from a lactating ewe (24 days) are shown in Fig. 2 and are in each case greater than those for the adult wethers; they were of the same order as those reported by McLean (1958). The higher apparent enzymic activities in the young lamb are consistent with the general hypothesis that some aspects of the carbohydrate metabolism of these animals differ from those of the adult sheep and more closely resemble those of non-ruminants (Jarrett & Potter, 1952; Jarrett & Filsell, 1960).

Some relationships of the relative functional status of pathways of metabolism to amounts of nicotinamide nucleotide coenzymes in the liver have been discussed by Caiger *et al.* (1962). It is apparent that a higher NAD concentration was present in the livers of prenatal and newborn lambs than in young lambs of both the present and previous studies (Table 1 and regression equation B). Since hypoxia may be one of the main dangers to the animal at this time (Dawes, 1961) a high proportion of NAD in relation to other nicotinamide nucleotide coenzymes may facilitate anaerobic glycolysis under conditions where anoxia limits the

reoxidation of NADH₂. The relative resistance of newborn lambs to hypoxia has been clearly demonstrated (Mott, 1961).

The change in the

$([NAD] + [NADH_2]) : ([NADP] + [NADPH_2])$ ratio during development of the sheep (Fig. 1) differs somewhat from that presented by Caiger *et al.* (1962). Whereas there were no measurements on foetal and newborn animals by Caiger *et al.* (1962), Fig. 1 shows that the coenzyme ratio is particularly high in the livers of animals at this stage of development, falling from 6–10 to 2–3 in the older lambs and adult sheep. This decline is similar to that which occurred in the young rat (Caiger *et al.* 1962), if allowance is made for the differences in the relative stage of maturity at birth of the lamb compared with the rat.

Calculations from the data of Nemeth & Dickerman (1960) indicate a similar fall in the coenzyme ratio in the liver of the guinea pig during development. Villem (1962) reported values for nicotinamide nucleotide coenzymes in human foetal liver and these indicated a coenzyme ratio of about 8. Roux *et al.* (1962) have determined these coenzymes in the liver of the rabbit during development but the change in coenzyme ratio cannot be calculated from the information available.

It would appear that a high coenzyme ratio is characteristic of mammalian liver in the prenatal and newborn animal, falling rapidly at about the time of birth and then attaining a characteristic ratio for adult liver. The changes in relative activities of enzymes associated with carbohydrate metabolism shown in the present work during growth of the lamb are consistent with the changing pattern of metabolism during development in ruminants.

SUMMARY

1. Specific enzymic reactions have been used to determine the concentration of both oxidized and reduced nicotinamide nucleotide coenzymes in the livers of foetal, newborn and young lambs and of adult sheep. The relationship between the rate of change of number of nuclei as a function of the rate of change of the amount of nicotinamide nucleotide coenzymes/nucleus during liver growth (Caiger *et al.* 1962) was confirmed.

2. The rates of reduction of NADP in the presence of glucose 6-phosphate, 6-phosphogluconate and glucose plus ATP were determined in liver homogenates. In each case these rates decreased during development of the lambs.

3. DNA phosphorus was determined in the livers of lambs and sheep and gave a mean value of 1.12 μ g. of DNA phosphorus/nucleus (range 0.93–1.30 μ g. in 19 animals).

4. The coenzyme ratio

$$([\text{NAD}] + [\text{NADH}_2]) : ([\text{NADP}] + [\text{NADPH}_2])$$

fell from 6–10 in the livers of prenatal and newborn lambs to 2–3 in older lambs and adult sheep.

5. These findings are discussed with reference to the growth and intermediary metabolism of the liver in lambs and sheep.

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The Biosynthesis of Coproporphyrinogen, Magnesium Protoporphyrin Monomethyl Ester and Bacteriochlorophyll by *Rhodospseudomonas capsulata*

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The biosynthesis of porphyrins and haem compounds in photosynthetic bacteria has been reviewed by Lascelles (1962). Conditions affecting the overall production of bacteriochlorophyll have also been studied, mainly by Lascelles (1956*a*, 1959, 1960, 1962), Cohen-Bazire, Sistrom & Stanier (1957), Tait & Gibson (1961), Gibson, Neuberger & Tait (1962*a*, *b*, *c*) and Sistrom (1962),

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but little is known of the later steps on the pathway leading to bacteriochlorophyll. The observation that mutant strains of *Chlorella*, which were unable to synthesize chlorophyll, produced protoporphyrin, protoporphyrin monomethyl ester, magnesium protoporphyrin, magnesium protoporphyrin monomethyl ester and magnesium vinyl phaeoporphyrin a_5 led Granick (1948*a*, *b*, 1950, 1961) to suggest that these compounds might be intermediates in the biosynthesis of chlorophyll