An interstrain difference in cholesterol synthesis in vitro in mice, dependent upon a difference in endogenous NADPH-generating capacity

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Abstract Earlier experiments have shown that significantly more endogenously generated NADPH is available for reduction of corticosterone in liver homogenates from C57BL/10 male mice than in those from the DBA/2 strain. To test the effect of this interstrain difference upon a representative NADPH-requiring biosynthetic pathway in vitro, the biosynthesis of cholesterol from mevalonic acid was studied in homogenates of livers from the two strains of mice, with and without addition of an NADPH-generating system. The incorporation of mevalonic acid into cholesterol in homogenates from the C57BL/10 strain is little affected by omission of the NADPH-generating system, but in the DBA/2 strain, addition of an NADPH-generating system is necessary to elevate the level of cholesterol synthesis to that of the C57BL/10 strain. Without this addition, the DBA/2 homogenate mainly produces lanosterol and other precursors of cholesterol which require NADPH for their further metabolism.

Supplementary key words NADPH · sterol biosynthesis · presqualene alcohol · C57BL/10 and DBA/2 mice

Recent studies in these laboratories have shown that corticosterone is metabolized by unsupplemented homogenates of livers from the C57BL/10J strain of mice several times more efficiently than by similar homgenates from the DBA/2J strain (1). The reduced metabolites of corticosterone obtained from both strains in such experiments were the same (with some quantitative differences), and the major difference between the liver homogenates of the two strains was the availability of endogenous NADPH. As evidence for this conclusion, the addition of an NADPH-generating system (glucose-6-phosphate, glucose-6-phosphate dehydrogenase, and NADP) to the liver homogenate of the C57 strain made very little difference to its efficiency of corticosterone metabolism, but addition of such an NADPH-generating system to the DBA liver homogenate stimulated its metabolism of the steroid to a level close to that of the C57 homogenate.

As a further test for the general significance of this difference, we have also examined cholesterol biosynthesis in liver homogenates of these two strains. We report that cholesterol synthesis in vitro by the C57 strain proceeds at high efficiency when its supply of NADPH derives solely from endogenous sources, but that, in contrast, in homogenates of the DBA strain this process depends heavily upon an exogenous supply of NADPH. In the absence of exogenous NADPH, liver homogenates of this strain accumulate large quantities of intermediates in the pathway of cholesterol synthesis, each of which requires NADPH for its further metabolism. One of these compounds appears to be the alcohol corresponding to the C_{30} pyrophosphate precursor of squalene, recently

Abbreviations: C57, C57BL/10J strain of mouse; DBA, DBA/2J strain of mouse; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; MVA, mevalonic acid.

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characterized by Epstein and Rilling and their coworkers (2–4) and synthesized by Altman, Kowerski, and Rilling (5).

In addition to studies of sterol biosynthesis, we have examined the stability of NADPH in liver preparations of these two strains of mice as a contribution to understanding the origin of their differing availabilities of NADPH. Results of these experiments are reported.

EXPERIMENTAL

Mice

C57BL/10J and DBA/2J mice were purchased from Jackson Laboratories, Bar Harbor, Maine. For some experiments, males were accommodated in our animal facility for several weeks and used when 9–11 wk old. Similar experiments were also done using 12–14-wk-old males from colonies started from breeders purchased from Jackson Laboratories and maintained in our animal facility (see Ref. 1 for details). Our experimental results were the same with mice from both sources.

Preparation of homogenates and incubation procedure

Mice were decapitated between 9 and 11 AM (after a period of 36 hr during which their cages were undisturbed), and the livers were removed quickly. Individual homogenates were prepared in ice-cold 0.08 M phosphate buffer, pH 7.4, essentially according to Bucher and McGarrahan (6) and were centrifuged for 10 min at 600 *g*. For incubations, 1-ml aliquots of the supernatant were added to 10-ml Erlenmeyer flasks containing 300 μg of DL-[5-^{3}H]mevalonic acid DBED salt (9.4–40.9 × 10^6 dpm; New England Nuclear Corp., Boston, Mass.) and ATP (3 μmoles) in 50 μl of buffer. When required, an NADPH-generating system (2 μmoles of NADP, 6 μmoles of glucose-6-phosphate, and 4 units of glucose-6-phosphate dehydrogenase) in 100 μl of buffer was added. Control incubations (minus NADPH-generating system) received the same volume of buffer. Incubations were carried out in air with shaking at 37°C for 3 hr (or shorter periods for the time-course studies). All incubations were terminated by addition of 1 ml of methanol and three pellets of potassium hydroxide and were allowed to stand first at 37°C for 1 hr, then overnight at room temperature. The nonsaponifiable material was extracted with redistilled ether, and the extracts were washed with water and dried with sodium sulfate. After evaporation of the ether under nitrogen, the residue was dissolved in hexane–ethyl acetate 3:1. A small aliquot of the nonsaponifiable fraction was assayed for radioactivity using a Packard liquid scintillation spectrometer; the external standard method was used to calculate the efficiency of counting.

Stability of NADPH in preparations of mouse liver

Homogenates of livers from six mice of each strain were prepared individually as described above and centrifuged at either 600 or 15,000 *g* for 20 min. The tetrasodium salt of NADPH (type II, Sigma Chemical Co., St. Louis, Mo.) was dissolved in 0.08 M phosphate buffer, pH 7.4 (6), at a concentration of 20 mg/ml, and 25 μl of this solution (equivalent to 0.35 mg of NADPH, as measured by UV absorbance at 340 nm) was added to 0.2-ml aliquots of the homogenate contained in screw-capped tubes. Control incubations were carried out with 0.2 ml of homogenate with addition of 0.25 μl of 0.08 M phosphate buffer, pH 7.4. The tubes were incubated in a shaking water bath at 37°C and were removed at intervals of 5, 10, 30, 60, and 120 min. The reactions were terminated by immediately adding 3 ml of spectrophotometric grade methanol to each tube, followed by agitation on a Vortex mixer. After centrifugation at 600 *g*, the clear supernatant was decanted into spectrophotometer cells and read at 340 nm against the appropriate blanks. The readings were a measure of NADPH remaining in the homogenate after the various time intervals. Spontaneous changes in the cofactor concentration were determined from the readings of the incubations of NADPH with buffer alone against a buffer blank. At the stated times the percentages of NADPH remaining in these control incubations were, respectively, 100, 98, 96, 93, and 87% of the initial amount. The values for the concentrations of NADPH remaining in the enzyme incubations were corrected accordingly.

Thin-layer chromatography

Silica gel G plates with layers approximately 0.35 mm thick were used for TLC, in some cases with the addition of 0.05% rhodamine 6G. The crude nonsaponifiable material was chromatographed in the solvent system hexane–ethyl acetate 3:1 by a triple development technique (7), in which the solvent front ran to 5 cm, then to 10 cm, and finally to 17 cm above the origin, with the plate removed from the tank and allowed to dry at room temperature between runs. TLC of other materials was carried out by the normal single development method. Known compounds were chromatographed on the edges of the plate and visualized either directly (with rhodamine 6G) or by charring with a hot wire. Radioactive peaks were localized using a Packard radiochromatogram strip scanner. Radioactive fractions were routinely extracted with ethyl acetate–methanol 9:1 once, and with hexane–ethyl acetate 3:1 twice. The extracts were passed through small columns of silica gel and sodium sulfate to remove the dye when rhodamine 6G plates were used, or otherwise through sodium sulfate alone. After TLC of fractions containing squalene and squalene oxide and

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the glycol and acetonide of squalene oxide, the oxide and its derivatives were recovered from the silica gel by extraction with methylene chloride in order to minimize problems arising from the sensitivity of these compounds to protonating solvents such as methanol.

Methods of radioassay and detection of radiolabeled materials on TLC plates have been described elsewhere (8).

Purification of sterols by bromination

For the purification of lanosterol acetate as its 24,25-dibromide, approximately 150 mg of the sterol acetate was dissolved in 4 ml of *t*-butanol, previously saturated with potassium bromide at 50–60°C, and bromine in *t*-butanol was added dropwise until the yellow color persisted. The solution was diluted with 0.3 ml of water and set aside at room temperature for 1 hr to allow crystallization of the dibromide, which was filtered off and recrystallized from ethyl acetate.[1]

Cholesterol was purified via the dibromide as described by Dempsey (10).

Gas–liquid chromatography

For most purposes, GLC was carried out using machines equipped with a hydrogen flame detector, with nitrogen as the carrier gas. Columns 6 ft × 1/8 inch were packed either with 5% diethylene glycol succinate polymer (DEGS) supported on silanized Chromosorb-W (100–120 mesh; Warner-Chilcott Laboratory, Instrument Division, Richmond, Calif.) or with 3% methyl-β-cyanoethylsiloxane polymer (XE 60) on the same support (Applied Science Laboratories Inc., State College, Pa.). These columns were operated at 200°C, with a nitrogen flow rate of 120 ml/min. For the characterization of the presqualene alcohol and its derivatives, a column 2 ft × 1/8 inch packed with 3% OV-1 on Chromosorb W-HP (Ohio Valley Specialty Co., Marietta, Ohio) was operated at 190°C (with the injector at the same temperature) with a nitrogen flow rate of 70 ml/min. All retention times are reported relative to cholestane (R_C values). The preparation of the trimethylsilyl ethers and acetates and the collection of the injected samples have been described (8).

Sources of pure cholesterol, lanosterol, squalene, and squalene 2,3-oxide have been described previously (8). Synthetic presqualene alcohol (2[2,6,10-trimethyl-1,6,9-undecatriene]-3-methyl-3-[4,8-dimethyl-3,7-nonadiene]-cyclopropylcarbinol) was generously made available to us by Dr. L. J. Altman of the Department of Chemistry, Stanford University.

[1] R. B. Clayton. Unpublished procedure (1956). (See also Ref. 9.)

RESULTS

Preliminary analysis of the nonsaponifiable metabolites of [^{3}H]mevalonic acid

The percentage of incorporation of labeled MVA into nonsaponifiable material in liver homogenates of C57 and DBA mice, incubated as described, with and without addition of an NADPH-generating system, are shown in Fig. 1. On TLC the distribution of labeling obtained with both strains (Fig. 2) with an NADPH-generating system added to the homogenate was essentially the same. Almost all of the radioactivity was present in a band corresponding in mobility to cholesterol (R_F 0.42), and similar results were obtained with the C57 in the absence of an added NADPH-generating system. For the DBA strain, however, omission of the NADPH-generating system led to the appearance of a large proportion of the label in regions of the chromatogram corresponding to products less polar than cholesterol which were absent or poorly represented in the other cases. The region (R_F 0.58) corresponding to lanosterol and presqualene alcohol was particularly prominently labeled, but significant labeling also appeared in the regions corresponding to squalene and squalene 2,3-oxide (R_F 0.92 and 0.86, respectively).

The values in Fig. 2 show that the distribution of label from [^{3}H]MVA between cholesterol and less polar products is heavily dependent upon exogeneous NADPH in the DBA strain, but much less so in the C57 strain.

Time course of labeling of nonsaponifiable fractions

The time course of incorporation of radioactivity from [^{3}H]MVA into each of the four fractions 2–5 (Fig. 2) is shown in Fig. 3. In both strains, with or without provision of an NADPH-generating system, radioactivity is incorporated into fraction 3 (later shown to consist predominantly of cholesterol) throughout the period of

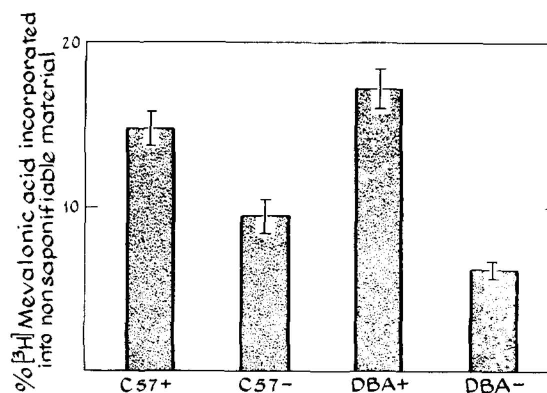

FIG. 1. Percentage of ^{3}H-labeled mevalonic acid (±SEM) incorporated into nonsaponifiable material in 10 C57 and 10 DBA liver homogenates. The symbols + and − indicate addition and omission of the NADPH-generating system, respectively.

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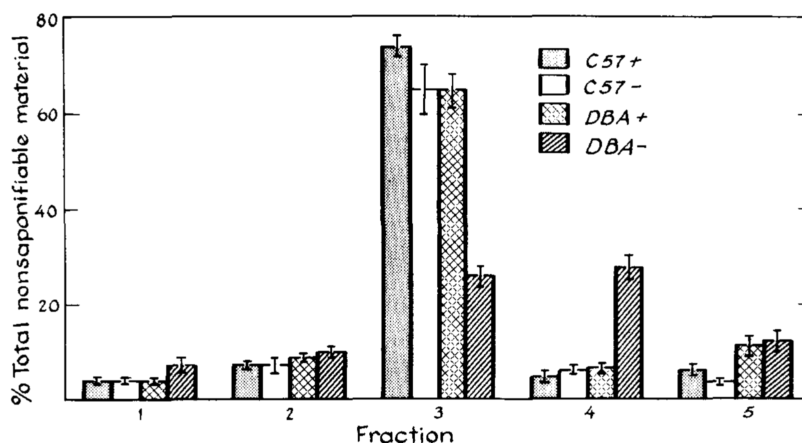

FIG. 2. Percentage of radioactivity (±SEM) in nonsaponifiable material (separated by TLC) from 10 3-hr incubations of liver homogenates from the two strains C57 and DBA, as described in the text. The symbols + and − indicate addition and omission of the NADPH-generating system, respectively. Fraction 1, R_F 0.02–0.15; fraction 2, R_F 0.15–0.33; fraction 3, R_F 0.33–0.48; fraction 4, R_F 0.48–0.64; fraction 5, R_F 0.64–0.96.

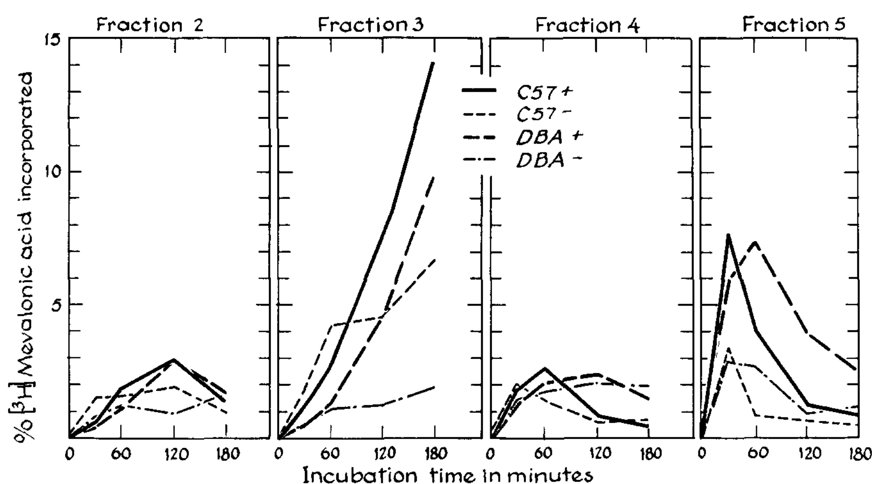

FIG. 3. Time course of labeling of fractions obtained from TLC of total nonsaponifiable material from three incubations of homogenates of liver from each of the two strains C57 and DBA, as described in the text. Incubations were stopped at 30, 60, 120, and 180 min. The symbols + and − indicate addition and omission of the NADPH-generating system, respectively.

study, while the amount of radioactivity in fractions 2, 4, and 5 declines during the second half of the experiment. Fractions 4 and 5 are shown below to comprise known precursors of cholesterol. Although fraction 2 was not examined further, its polarity and time course of labeling suggest that it also consists of precursors of cholesterol: possibly materials in which the methyl groups of lanosterol are partially oxidized (11, 12). The dependence of cholesterol formation upon the addition of an NADPH-generating system is especially marked in the case of the DBA incubations, but is much less for C57.

Further analysis of nonsaponifiable products

Further detailed analyses were carried out on the components of fractions 3–5 obtained from 3-hr incubations as indicated in Fig. 2, with particular attention to products from incubations of the DBA strain without exogenous NADPH.

Fraction 3. This material had the mobility on TLC of cholesterol (R_F 0.42) in the system hexane–ethyl acetate 3:1. Portions of this product containing 11,500–98,400 dpm derived from liver incubations of the two strains, with and without the addition of an NADPH-generating system, were analyzed by GLC on XE 60. Fractions of the effluent material were collected continuously during intervals of 1 min and were assayed for radioactivity. In all cases except that of DBA without added NADPH, 69–80% of the radioactivity coincided with the mass peak of cholesterol (R_C 5.7), and the remainder emerged over a range of longer retention times (R_C 6.2–10.0), prob-

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ably corresponding to a mixture of Δ^7-, $\Delta^{5,7}$-, and Δ^{24}-sterols, which are known precursors of cholesterol (13). The percentage of radioactivity associated with the cholesterol peak from DBA homogenates not reinforced with an NADPH-generating system was lower (57%).

Portions of this labeled material derived from incubations from each strain were mixed with pure cholesterol, which was then purified via the dibromide and finally recrystallized. Radioassay of the resulting crystals indicated marked losses of labeled material. In each case, repetition of this procedure verified that a constant specific activity (±5%) has been attained during the first purification sequence. The results of both the GLC analysis and the bromination procedure are shown in Table 1.

Fraction 4. On the assumption that fraction 4 consisted of lanosterol and closely related sterols, its analysis was attempted by GLC of the trimethylsilyl ether derivatives on DEGS. Erratic results indicated decomposition of the material, and this approach was abandoned.

More satisfactory GLC analyses of samples of these materials in the free form containing 26,000–44,500 dpm were made with the use of XE 60. When the fraction was obtained from incubations to which the NADPH-generating system was added, the radioactivity was recovered almost entirely in association with two peaks. One of these was broad (R_C 6.5), suggesting that this material consisted of a mixture of dihydrolanosterol (R_C 7.2), Δ^7-4α-methylcholestenol (lophenol) (R_C 7.2), and Δ^8-4α-methylcholestenol (R_C 6.3). This material was not examined further. The second peak coincided in retention time (R_C 8.7) with lanosterol. In the DBA material, the ratio of radioactivity associated with these peaks (R_C 8.7:R_C 6.5) was 0.71; in the C57 material this ratio was 0.43. When the NADPH-generating system was omitted, the radioactive peak, R_C 6.5, was absent and the activity emerged in association with lanosterol and a new broad band (R_C 1.1) in both strains. The behavior of the latter material suggested a decomposed product, and it was found to correspond to presqualene alcohol (2–5) under similar GLC conditions. These results are summarized in Table 2.

We undertook a more accurate analysis of the amount of material corresponding to presqualene alcohol in fraction 4 from incubations of DBA liver without addition of the NADPH-generating system, since only this fraction contained it in significant amount. An aliquot of fraction 4 containing 248,000 dpm on TLC in carbon tetrachloride–ether 4:1 gave two major radioactive fractions corresponding to lanosterol, R_F 0.25 (66,300 dpm, 26.7%), and presqualene alcohol, R_F 0.34 (116,900 dpm, 47%). An aliquot of the fraction with an R_F of 0.34 (30,000 dpm) was chromatographed on OV-1, and the collected radioactivity (15,000 dpm) coincided entirely with the mass peak of presqualene alcohol, R_C 1.80. Another portion of the material (25,600 dpm) was acetylated and also chromatographed on OV-1 with recovery of 13,000 dpm in association with the mass peak of the acetate of presqualene alcohol (R_C 2.4). These recoveries (50%) are somewhat higher than the usual recovery of 40% obtained on 6-ft columns of XE 60. The results suggest that the fraction with R_F 0.34 is predominantly presqualene alcohol.

A portion of the more polar fraction (R_F 0.25) was acetylated, and a sample of the acetylated product containing 57,800 dpm was mixed with 145.3 mg of pure lanosterol acetate, which was then purified as the dibromide. The product on crystallization had a specific activity of 270 dpm/mg, which was unchanged by three further recrystallizations and indicated the presence of 80% of the radioactivity in lanosterol.

TABLE 1. Radioactivity in fraction 3 found as cholesterol

	C57+[a]	C57−	DBA+	DBA−
GLC, XE 60				
Injected dpm	72,800	89,600	98,400	11,500
Percentage recovered[b] with mass peak of cholesterol	76	80	69	57
Purification via dibromide				
Theoretical specific activity[c] (dpm/mg)	1,300	1,567	4,040	492
Final specific activity[d] (dpm/mg)	940	1,363	2,267	120
Percentage cholesterol	72	87	56	24

[a] The designations + and − indicate addition and omission of an NADPH-generating system, respectively.
[b] Corrected for an efficiency of recovery of 40%.
[c] Calculated on the assumption that all the labeled material was cholesterol.
[d] Following admixture with authentic cholesterol and two passages through the bromination–debromination procedure.

TABLE 2. Distribution of radioactivity in various components of fraction 4, as shown by GLC on XE 60

	C57+[a]	C57−	DBA+	DBA−
Injected dpm	44,500	29,100	26,500	37,800
Percentage recovered[b] with mass peaks corresponding to:				
Presqualene alcohol (R_C 1.1)	0	45	0	57
Lanosterol (R_C 8.7)	30	22	40	38
Mixed products (R_C 6.5), presumed to be mainly dihydrolanosterol and 4α-methyl sterols	70	<3	56	<1

[a] The designations + and − indicate addition and omission of an NADPH-generating system, respectively.
[b] Corrected for an efficiency of recovery of 40%.

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Fraction 5. Since some incubations yielded material which showed two radioactive peaks on TLC in the region of fraction 5 corresponding to squalene (R_F 0.92) and squalene 2,3-oxide (R_F 0.86), the material from fraction 5 was analyzed further by TLC in the solvent system hexane–ethyl acetate 19:1, which gave a better resolution of these compounds (R_F squalene, 0.72; R_F squalene 2,3-oxide, 0.38). The results are shown in Table 3. Aliquots of the fraction that had the mobility of squalene were further analyzed by GLC on DEGS, with collection of emergent radioactive materials which in each case were almost entirely associated with the mass peak of squalene (R_C 1.8) (Table 3).

The characterization of squalene 2,3-oxide by direct GLC is unsatisfactory because of its decomposition (8). Samples of the material with R_F 0.38 obtained from incubations of each strain in the presence of exogenous NADPH were mixed with 60 μg of unlabeled squalene 2,3-oxide in each case and hydrolyzed to give the corresponding 2,3-dihydro-2,3-glycol (8). The products were analyzed by TLC in the system hexane–ethyl acetate 3:1 on plates impregnated with rhodamine 6G. Fractions corresponding to squalene 2,3-oxide (R_F 0.62) and 2,3-dihydrosqualene 2,3-glycol (R_F 0.20) were recovered and assayed for radioactivity. Although the unlabeled oxide was entirely converted to glycol by this procedure (as evidenced by the visible distribution of mass on the TLC plate), a significant portion of the labeled material failed to react (Table 3) and was inert on a second attempt at hydrolysis. Most probably this unreactive material was the corresponding 3-ketone, which we have noted on other occasions to form from the labeled oxide during storage and to have the same R_F as the oxide in most TLC systems (14). For further characterization, the labeled glycol from each source was converted to the acetonide (15), which was analyzed by TLC. The radioactivity was associated entirely with the acetonide band, which was recovered and analyzed by GLC on DEGS. Essentially all of the label was associated with a single peak corresponding to the acetonide. The details of these analyses are shown in Table 3.

Data for the incorporation of MVA into different components of the nonsaponifiable fraction under various incubation conditions are summarized in Table 4.

Stability of NADPH in mouse liver preparations

The evident difference in the availability of endogenously generated NADPH in C57BL/10 and DBA/2 mice could reflect a corresponding difference in the capacity of liver tissues of the two strains to generate NADPH or in the demand of competing pathways for its utilization. This question was examined in experiments (Fig. 4) in which the stability of NADPH in two types of liver preparations from the two strains were measured. One ex-

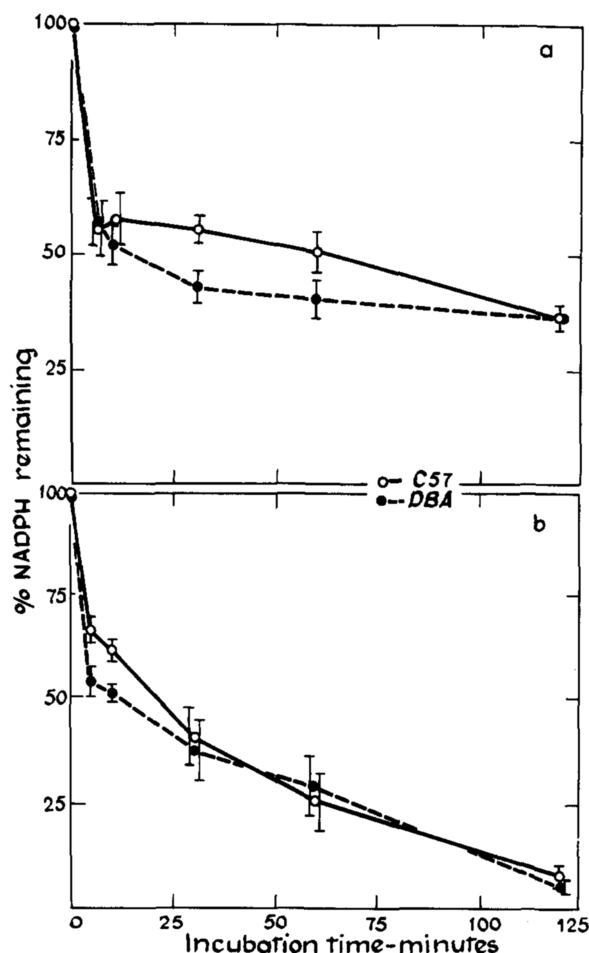

FIG. 4. Stability of NADPH in preparations from liver homogenates of C57 and DBA mice. The figure shows the disappearance of NADPH in supernatants obtained by centrifugation of homogenates at 600 *g* (*a*) and at 15,000 *g* (*b*). Other conditions are described in the text.

periment (Fig. 4*a*) utilized a homogenate after centrifugation at 600 *g* as employed in the experiments with mevalonic acid reported here. The other (Fig. 4*b*) made use of the supernatant of a homogenate after centrifugation at 15,000 *g*, such as we have used for corticosterone metabolism (1). The results show that in both of these preparations there was significantly (P = 0.05) less NADPH remaining in the DBA at the 30-min interval in the 600 *g* supernatant and at the 5- and 10-min intervals in the 15,000 *g* supernatant. At no other times examined during the 2-hr incubation period was a significant interstrain difference found for either type of preparation.

DISCUSSION

Earlier work in these laboratories has shown (1) that the inbred strains of mice, DBA/2 and C57BL/10 differ profoundly in their availability of NADPH for in vitro

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TABLE 3. Composition of fraction 5

Analytical Procedure and Conditions	C57+[a]	C57−	DBA+	DBA−
(*1*) TLC (hexane–ethyl acetate 19:1)				
Initial ^3H content of sample	350,000[b]	84,000	680,000	223,000
^3H as squalene 2,3-oxide (R_F 0.38)	161,000 (46)	12,800 (15)	393,000 (58)	41,700 (19)
^3H as squalene (R_F 0.72)	158,000 (45)	58,000 (69)	194,000 (29)	145,000 (65)
(*2*) GLC of squalene fraction (R_F 0.72) from (*1*) on DEGS				
^3H content of injected sample	46,400	15,000	40,300	35,200
Percentage recovered[c] with mass peak of squalene (R_C 1.8)	86	87	96	94
(*3*) Conversion of squalene 2,3-oxide fraction (R_F 0.38) from (*1*) to 2,3-glycol and TLC (hexane–ethyl acetate 3:1)				
^3H content of sample	35,700		148,000	
^3H as 2,3-glycol (R_F 0.2)	15,020 (42)		84,400 (57)	
^3H unreacted[d] (R_F 0.62)	12,700 (36)		34,000 (23)	
(*4*) Conversion of 2,3-glycol from (*3*) to acetonide and TLC (hexane–ethyl acetate 4:1)				
^3H content of sample	9,870		39,300	
^3H as acetonide (R_F 0.66)	9,570 (97)		38,500 (98)	
(*5*) GLC of acetonide from (*4*) on DEGS				
^3H content of injected sample	4,900		19,600	
Percentage recovered[c] with mass peak of 2,3-acetonide (R_C 5.38)	100		100	

[a] Designations + and − indicate addition and omission of an NADPH-generating system, respectively.
[b] Values are given as dpm. Figures in parentheses indicate percentages of initial fractions represented by the stated recoveries of labeled material.
[c] Percentages of recovered materials after GLC are corrected for a collection efficiency of 45%.
[d] This unreacted material is assumed to be the 3-ketone isomer of squalene 2,3-oxide, formed artifactually (see text).

TABLE 4. Incorporation of [^{3}H]mevalonic acid into components of the nonsaponifiable fraction in liver homogenates of C57 and DBA mice

	C57+[a]		C57−		DBA+		DBA−	
	% of Nonsap.[b]	% of Avail. MVA[c]	% of Nonsap.	% of Avail. MVA	% of Nonsap.	% of Avail. MVA	% of Nonsap.	% of Avail. MVA
Cholesterol[d]	53.4	7.69	56.6	5.43	36.1	6.17	6.3	0.40
Lanosterol	1.6[e]	0.23[e]	1.3[e]	0.12[e]	2.4[e]	0.41[e]	5.8[d]	0.37[d]
Dihydrolanosterol and 4α-methyl sterols[e]	3.6	0.24	<0.2	<0.02	3.6	0.58	0.3	<0.02
Squalene 2,3-oxide	2.9[f] (1.2)[e]	0.42[f] (.17)[e]	0.5[f]	0.05[f]	6.4[f] (3.7)[e]	1.09[f] (0.63)[e]	2.3[f]	0.14
Squalene[e]	2.6	0.37	2.2	0.21	3.1	0.53	7.3	0.46[f]
Presqualene alcohol[e]	0	0	2.7	0.26	0	0	12.9	0.81

[a] The designations + and − indicate addition and omission of an NADPH-generating system, respectively.
[b] Percentage of total nonsaponifiable fraction accounted for as the indicated compound.
[c] Percentage of available [^{3}H]mevalonic acid incorporated into the indicated fraction.
[d] Calculated from the results of dibromide purification.
[e] Calculated on the basis of recovery of labeled material from GLC (squalene 2,3-oxide analyzed as the 2,3-acetonide).
[f] Values calculated from fractions recovered from TLC.

reductive metabolism of corticosterone in liver homogenates. Since NADPH is central to many important synthetic processes, this finding raises questions concerning its possible physiological implications. Several cellular processes might be less efficient in the DBA strain than in the C57 in proportion to their demand for NADPH. One of these is the biosynthesis of cholesterol, and the present results show that, like the metabolism of corticosterone, this metabolic pathway depends only minimally upon exogenous NADPH in liver homogenates of the C57 strain, but is heavily dependent upon it in the DBA. The overall levels of incorporation of [^{3}H]MVA into nonsaponifiable lipids and the patterns of its distribution in various fractions are similar in both strains in the presence of an added NADPH-generating system. In the C57 homogenates without added NADPH, incorporation of MVA is reduced by about one-third, but in the DBA without exogenous NADPH, the incorporation is reduced by about two-thirds of its level with the added cofactor. In the absence of added NADPH, the C57 homogenate produces 71% of the amount of cholesterol and 52% of the amount of lanosterol that it yields when the cofactor is added. In the DBA strain, by far the greatest deficit in product formation concerns cholesterol

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itself, which is formed in only about 6.5% of the yield that is obtained when the NADPH-generating system is present. In contrast, the amount of lanosterol produced is essentially the same as under optimal conditions. This relative difference is presumably a reflection of the grossly different requirement for NADPH in lanosterol formation from mevalonate (12) (2 equivalents) and in the conversion of lanosterol to cholesterol (approximately 14 equivalents).

The accumulation in DBA homogenates lacking exogenous NADPH of a compound that is probably identical with "presqualene alcohol," recently identified as a precursor (in the form of its pyrophosphate) of squalene (2–5), is a reasonable finding, since this compound requires NADPH for its further metabolism. Presumably, the formation of the free alcohol under our conditions reflects the activity of hepatic phosphatases.

While the results reported here are entirely consistent with our earlier findings, it should be emphasized that our observations with respect to cholesterol synthesis are limited to cell-free preparations and that further work is required to test their in vivo significance. Such studies may be particularly interesting, since the literature on inbred strains of mice contains a number of references to important ways in which the C57BL and DBA/2 strains differ, e.g., in brain weight:body weight ratio, longevity, alcohol tolerance, drug effects, and behavioral and reproductive characteristics (16). Differences in NADPH availability may contribute to such physiological differences in a variety of ways that invite speculation and further experimentation.

Whether the interstrain difference in availability of NADPH results from a difference in its production or in its consumption by competing pathways, or from a combination of these factors, can only be determined by further detailed experiments. After an initial phase during which NADPH is consumed significantly faster in the DBA preparations (Fig. 4), the stability of the cofactor in liver preparations from the two strains becomes the same. During this later period, both cholesterol synthesis (Fig. 2) and corticosterone reduction proceed actively, and it is therefore improbable that differences in the rates of these processes reflect differences in stability of NADPH. Moreover, the interstrain difference in NADPH consumption for corticosterone reduction found in our earlier experiments (1) is about three times greater than the maximal difference in NADPH consumption by competing reactions in a similar enzyme system (15,000 *g* supernatant, Fig. 4*b*). In the case of MVA metabolism in a 600 *g* supernatant preparation, however, the maximal interstrain difference in competing NADPH utilization (i.e., at 30 min, Fig. 4*a*) probably approximates the interstrain difference in overall availability of the cofactor for sterol synthesis. On the other hand, corticosterone reduction occurs in the absence of added NADPH equally efficiently in both types of preparations from a given strain.[2]

Since these comparisons are subject to various uncertainties, we can only conclude that the results of tests of the stability of NADPH in these liver preparations do not point to gross interstrain differences that could readily account for the differences in NADPH availability. An explanation based primarily upon a difference in endogenous NADPH-generating capacity thus seems more likely, and this is supported by the finding that the levels of microsomal and soluble glucose-6-phosphate dehydrogenases in the C57 are 50% and 25% higher, respectively, than in the DBA.[2] The former enzyme exists as different electrophoretic variants under the control of different alleles of the autosomal locus *Gpd-1* in several C57 strains on the one hand, and the strains DBA/1 and DBA/2 on the other (17, 18).

Further work on these and related enzyme differences between these two strains of mice is in progress. Our findings may be of value in the genetic analysis of the interplay of factors determining the availability of NADPH and other physiological and biochemical characters with which it is involved.

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