Biochemical Pharmacology, Vol. 24, pp. 2279–2285. Pergamon Press, 1975. Printed in Great Britain.

STUDIES ON THE *IN VITRO* TURNOVER OF HISTONE ACETYL GROUPS AND THE INCREASE OF RNA SYNTHESIS IN RAT LIVER NUCLEI AFTER *IN VIVO* APPLICATION OF α-HEXACHLOROCYCLOHEXANE (α-HCH)

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(*Received* 15 *October* 1974; *Accepted* 26 *June* 1975)

Abstract—Nuclear uptake of radioactive acetate into rat liver histones *in vitro* peaked at 2 and 24 hr after α-HCH application, when control values were exceeded by approximately 200%. The magnitudes of acetate uptake into histone fractions of rats treated with α-HCH for 24 hr and of control rats were in the following order: F3 > F2b > F2a1 > F2a2. Retention of radioactive acetate in F3 and F2a1 histone fractions of rats pretreated with α-HCH for 24 hr accounted for 61% of the radioactive acetate retained in total histones, which was nearly seven times higher than in histones of control rats. Preincubation of rat liver nuclei isolated 24 hr after α-HCH administration with acetylcoenzyme A resulted in an enhancement of the already α-HCH stimulated RNA synthesis. This, as well as the coincidence of maximally increased acetate content in histones and increased nuclear RNA synthesis 24 hr after α-HCH, suggests a connection between the two nuclear processes. No effect of preincubation with acetylcoenzyme A was seen on RNA polymerase activities in liver nuclei from rats pretreated for 2 hr with α-HCH which also contain highly acetylated histones or in liver nuclei from control rats. So it is assumed that beside the increase of histone acetate content additional conditions need to be fulfilled to start and to guarantee the α-HCH induced gene activation in rat liver.

Biochemical alterations in rat liver after application of α-hexachlorocyclohexane (α-HCH) offer an attractive model for studying processes involved in changes of genetic restriction associated with the induction of several enzymes [1, 2] as well as with the prereplicative phase of liver proliferation.

Recently we demonstrated that application of α-HCH to rats leads to an increase of nuclear endogenous RNA polymerase activities [3] and to an enhanced capacity of rat liver chromatin to serve as a template for heterologous RNA polymerase [4]. Since a connection has been claimed between enzymatic histone side-chain modifications and availability of DNA sequences in chromatin for RNA synthesis [5, 6] we studied the temporal relationship between histone acetylation and RNA synthesis after α-HCH treatment. The peaks of elevated histone acetylation at 2 and 24 hr after α-HCH preceded and accompanied increased nuclear RNA polymerase activities [3].

The present paper deals with the changes in nuclear *in vitro* turnover of acetyl groups in rat liver histone fractions caused by α-HCH and describes a connection between increased nuclear *in vitro* histone acetylation and increased nuclear *in vitro* RNA synthesis after α-HCH application.

MATERIALS AND METHODS

Animals. Female albino Wistar rats weighing 150 g received 200 mg α-HCH dissolved in rape seed oil per kg body wt via a stomach tube as described in [3]. Control rats were treated with corresponding volumes of rape seed oil. Animals were killed in all experiments between 8:00 and 10:00 a.m.

Rat liver nuclei were isolated as described previously [7] using 0·32 M sucrose with 3 mM $MgCl_2$, 20 mM Tris–HCl pH 7·4. The Triton-X-100 treatment was omitted.

Chromatin was prepared according to Wilhelm *et al.* [8].

In vitro *assay for nuclear histone acetylation.* Histones were isotopically labelled by incubating isolated liver nuclei (2 mg DNA) from α-HCH-treated and control rats with 18·5 μCi [^{3}H]acetylcoenzyme A (sp. act. 0·92 Ci-m-mole, NEN Chemicals) in 0·32 M sucrose containing 3 mM $MgCl_2$ and 20 mM Tris–HCl pH 7·4 at 37°. The reaction was stopped either by adding an excess of ice-cold H_2SO_4 or by pipetting 0·5-ml aliquots of the incubation assay into ice-cold H_2SO_4 up to a final concentration of 0·4 N. For determination of [^{3}H]acetate retention in histones an excess of unlabelled acetylcoenzyme A was added to the incubation assay at 20 min when histones were maximally ^{3}H-acetylated and the reaction was further incubated for 40 min and terminated by adding ice-cold H_2SO_4 up to 0·4 N. The [^{3}H]acetate retention after the 40 min 'chase' period provides an indication of histone deacetylase activities for the different histone fractions.

Determination of acetate incorporation and retention in histones by double labelling. Liver nuclei (12 mg

DNA) from rats treated with α-HCH for 24 hr and from control rats were incubated with saturating amounts of either [^{3}H]acetylcoenzyme A (sp. act. 58 mCi/m-mole) or [1-^{14}C]acetylcoenzyme A (sp. act. 58 mCi/m-mole) in 0·32 M sucrose containing 3 mM $MgCl_2$ and 20 mM Tris–HCl pH 7·4 at 37°. After 20 min of incubation, when histones were acetylated up to maximum values, an excess of unlabelled acetylcoenzyme A was added and the reaction mixture was further incubated for 40 min. At 20 and 60 min of incubation aliquots of the differentially labelled rat liver nuclei were taken for the preparation of chromatin which was depleted of histone fractions F1, F2b, F2a2 essentially as described by Bolund and Johns [9]. From the partially depleted chromatin (protein/DNA ratio = 0·43 ± 0·04) the arginine-rich histone fractions F3 and F2a1 were extracted with 0·4 N H_2SO_4. Equal amounts of protein of ^{14}C-labelled F3 and F2a1 fractions from control rats dissolved in 0·9 N acetic acid were mixed either with ^{3}H-labelled F3 and F2a1 fractions from the same control rats or with ^{3}H-labelled F3 and F2a1 fractions from α-HCH treated rats. Electrophoresis of the mixtures, staining, destaining, scanning, slicing and radioanalyzing of the gels was the same as described below. ^{3}H- and ^{14}C-radioactivity in each 1 mm gel slice was determined by using the absolute activity analyzer model 544 attached to the Packard Liquid Scintillation Counter 3380.

Histones were extracted from purified nuclei or chromatin with ice-cold 0·4 N H_2SO_4 twice for 30 min. The arginine-rich fractions F3 and F2a1 were extracted selectively with 0·4 N H_2SO_4 from chromatin which had been depleted of F1, F2b, F2a2 according to the method of Bolund and Johns [9] by treatment with ion-exchange resin AG 50W-X2 (mesh 200–400, Bio-Rad, Richmond, CA, U.S.A.). In order to reduce degradation of histones by proteases 6 mM sodium bisulphite was added to all the nuclear and chromatin preparations which served as starting material for histone extraction. The distribution of [^{3}H]acetate in the various histone fractions was examined by separating equal amounts of histones on polyacrylamide gels under identical electrophoretic conditions followed by gel slicing and scintillation counting.

Polyacrylamide gel electrophoresis and radioanalysis of histones. Electrophoresis was carried out essentially as described by Panyim and Chalkley [10]. The gels (13 × 0·6 cm) contained 15% acrylamide and either 2·5 M urea at pH 2·7 or 3·125 M urea at pH 2·8 or 6·25 M urea at pH 3·2. Ethylene diacrylate was used as cross-linking agent instead of methylene bisacrylamide. Other modifications and details are given in the legends of the figures (Figs. 3a, b). Histone samples were dissolved at a final concentration of 1 mg/ml in 0·9 N acetic acid containing 15% sucrose. Gels were stained in a solution containing 0·1% amido black 10B (E. Merck, Darmstadt, Germany) 7% acetic acid–20% ethanol (v/v), destained with 7% acetic acid–35% ethanol (v/v). For destaining, gels were placed in an apparatus constructed in our workshop by which the dye was removed from the gels electrophoretically perpendicular to the direction of separation as well as by diffusion. Continuous circulation of the cooled destaining solution which was achieved by pumps and a separate cooling system shortened the time for destaining 'histones' gels considerably without the unwanted loss of histone fractions during electrophoretic destaining [11]. Following destaining, absorbance profiles of the banding patterns were determined at 600 nm using a Gilford spectralphotometer model 240 with scanning attachment. Gels were cut either into 1-mm slices or histone bands were selectively sliced using a technique described elsewhere [12]. Gel slices were depolymerised by 30% H_2O_2 and dissolved in Bray's scintillation solution [13] containing 1% Nuclear-Chicago-Solubilizer (NCS, Amersham/Searle, IL, U.S.A.).

RNA polymerase assay was carried out essentially as described by Widnell and Tata [14] and contained the following components in a final volume of 1 ml:

(a) RNA polymerase I (or A) measured at low ionic strength, pH 8·5: 0·2 ml nuclei suspension (about 50 μg DNA), 6 mM $MgCl_2$, 100 mM Tris–HCl, 0·25 mM EDTA, 6 mM NaF, 10 mg bovine serum albumin, 0·5 mM ATP, GTP, CTP, 7·3 μCi [^{3}H]UTP (sp. act. 90 mCi/m-mole).

(b) RNA polymerase II (or B) measured at high ionic strength pH 7·5: 0·2 ml nuclei suspension (about 50 μg DNA), 3 mM $MnCl_2$, 100 mM Tris–HCl, 0·25 mM EDTA, 325 mM $(NH_4)_2SO_4$, 10 mg bovine serum albumin, 0·5 mM ATP, GTP, CTP, 7·3 μCi [^{3}H]UTP (sp. act. 90 mCi/m-mole).

The reaction was started with 0·2 ml nuclear suspension, incubated 3 min at 37° and stopped by adding 5 ml ice-cold 0·2 N perchloric acid (PCA) with 50 mM $Na_2P_2O_7$. After adding 100 μl of 1% solution of serum albumin the precipitate was collected by centrifugation, washed three times, each time with 5·0 ml ice-cold PCA. DNA was hydrolyzed with 0·5 N PCA at 75° for 15 min and aliquots (0·2 ml of the DNA extract) were placed into scintillation vials containing 10 ml of Bray's scintillation mixture [13].

Radioactivity was determined in a Packard Liquid Scintillation Counter model 3380 using external standardisation. Incorporation of radioactivity into total histones was determined by the paper disc method described by Sekeris *et al.* [15].

DNA was assayed according to the method of Burton [16] using calf thymus DNA as standard.

Protein was determined by the method of Lowry *et al.* [17] using bovine serum albumin as standard.

RESULTS

In order to determine whether the described peaks of increased rat liver histone acetylation at 2 and 24 hr after α-HCH administration [3] are related to an increased acetate uptake and a changed or unchanged acetate release from histones, the kinetics of nuclear *in vitro* histone acetylation in rat liver were studied at different times after α-HCH application.

The uptake of [^{3}H]acetate rose linearly in both α-HCH treated and control groups up to maximum values at 20 min when 2, 3 and 3·5 times the control level were reached in histones isolated 0·5, 2 and 24 hr after α-HCH application (Fig. 1). Between 20 and 60 min of incubation the amounts of radioactive acetate incorporated in liver histones decreased continuously but at different rates in α-HCH treated and control groups. The histone-bound [^{3}H]acetate of α-

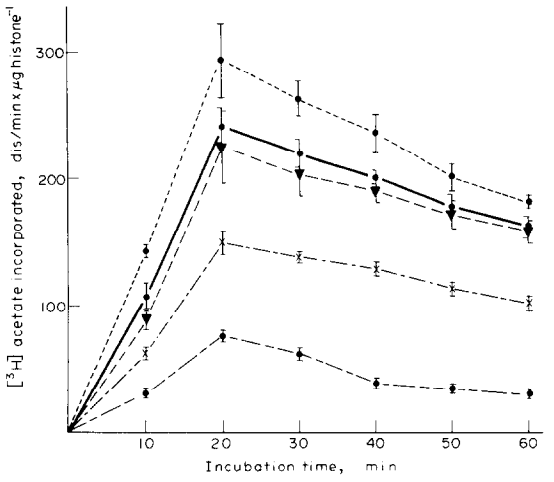

Fig. 1. Time course of *in vitro* [^{3}H]acetate incorporation in total histones by rat liver nuclei of control and α-HCH treated rats. Isolated nuclei (2 mg DNA) from α-HCH treated and control rats were incubated with 18·5 µCi [^{3}H]acetylcoenzyme A (spec. act. 0·92 Ci/m-mole) in 0·32 M sucrose containing 3 mM $MgCl_2$ and 20 mM Tris–HCl pH 7·4 at 37°. At the times indicated in Fig. 1 aliquots of the incubation assay were pipetted into ice-cold H_2SO_4 up to a final concentration of 0·4 N. Extraction of the histones from the aliquots and determination of incorporated radioactivity are described under Methods. Each point represents the arithmetic mean ± S.D. of histone specific activities derived from three experiments with two animals each. Control rats (●—●) and rats pretreated for 30 min (×----×), 60 min (▼---▼), 2 hr (●——●) and 24 hr (●----●) with 200 mg α-HCH/kg body wt.

HCH treated groups as compared to that of control groups is much greater if the ^{3}H-radioactivity is determined at 60 min rather than at 20 min of incubation. This could indicate differences in the release of

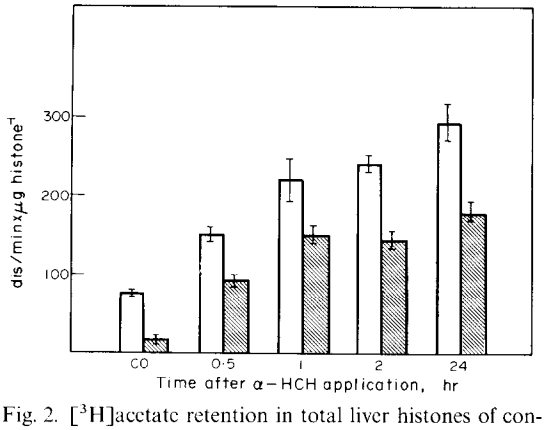

Fig. 2. [^{3}H]acetate retention in total liver histones of control and α-HCH treated rats. Histones were labelled up to maximum values by incubating rat liver nuclei (2 mg DNA) with 18·5 µCi [^{3}H]acetylcoenzyme A (sp. act. 0·92 Ci/m-mole) for 20 min (□) in 0·32 M sucrose containing 3 mM $MgCl_2$ and 20 mM Tris HCl pH 7·4 at 37°. After further incubation of nuclei at 37° in the presence of a 40-fold excess of unlabelled acetylcoenzyme A for 40 min, reaction was stopped by adding ice-cold H_2SO_4 up to a final concentration of 0·4 N and ^{3}H-radioactivity retained in total histones (▨) was determined as described under Methods. Each value represents the arithmetic mean ± S.D. from four experiments with two rats each.

[^{3}H]acetate groups from histones of α-HCH treated and control rats but had to be tested under conditions which eliminate a reiterated incorporation of [^{3}H]acetyl groups as might occur at longer incubation times in the kinetic experiments of Fig. 1.

Therefore the retention of [^{3}H]acetyl groups in prelabelled rat liver histones was determined in pulse chase experiments at different times after α-HCH application. Figure 2 shows that the retention of newly incorporated [^{3}H]acetate at the end of the chase period was remarkably greater in the α-HCH treated groups. After a 40-min chase period 60% and 60·9% respectively of the maximum [^{3}H]acetate uptake was retained in histones by liver nuclei isolated 2 and 24 hr after α-HCH application compared to 25% in the control groups. Nevertheless, considerably more [^{3}H]acetate was released during the chasing period from rat liver histones isolated 2 and 24 hr after α-HCH than from histones of control rats or of rats pretreated with α-HCH for 0·5 or 1 hr (Figs. 1 and 2). Since this could be caused by more or less specific deacetylases [18, 19] acting on histone fractions ace-

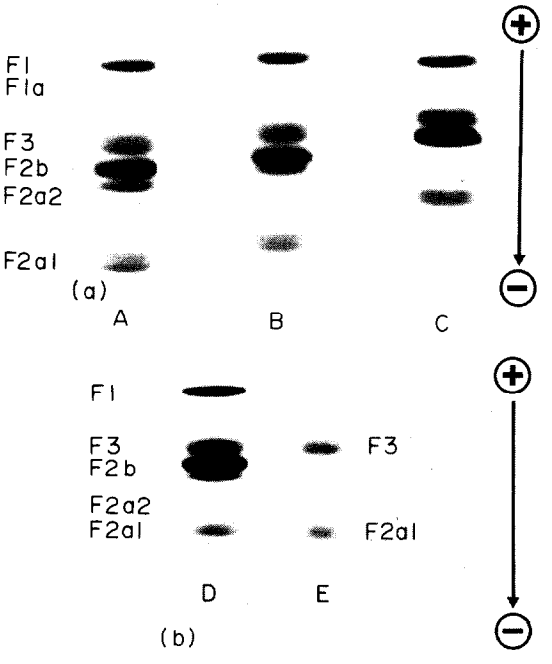

Fig. 3. (a) Effect of different urea concentration in polyacrylamide gels on the resolution of F3, F2b, F2a2 histone fractions. After pre-electrophoresis at 1 mA/gel for 12 hr electrophoresis was performed at 2 mA/gel (0·6 × 13 cm) for 5 hr on 15% polyacrylamide gels [16] under the following conditions: (A) 3·125 M urea, 0·9 N acetic acid, pH 2·8, (B) 2·5 M urea, 0·9 N acetic acid, pH 2·7, (C) 6·25 M urea, 0·9 N acetic acid, pH 3·2. Note the separation of F2a1 histone into two bands which are the acetylated and non-acetylated fraction of F2a1 and note fraction F1a the acetylation of which will be discussed later. (b) Polyacrylamide gel electrophorctic patterns of histones extracted with 0·4 N H_2SO_4 from isolated rat liver chromatin: (D) histones from rat liver chromatin, (E) arginine-rich histone fraction F3, F2a1 from rat liver chromatin depleted of histone fraction F1, F2b, F2a2 by treatment with ion-exchange resin AG 50W-X2 (mesh 200–400) in 0·45 M NaCl, 0·05 M sodium phosphate buffer, 4 M urea pH 7·0 according to Bolund and Johns [15].

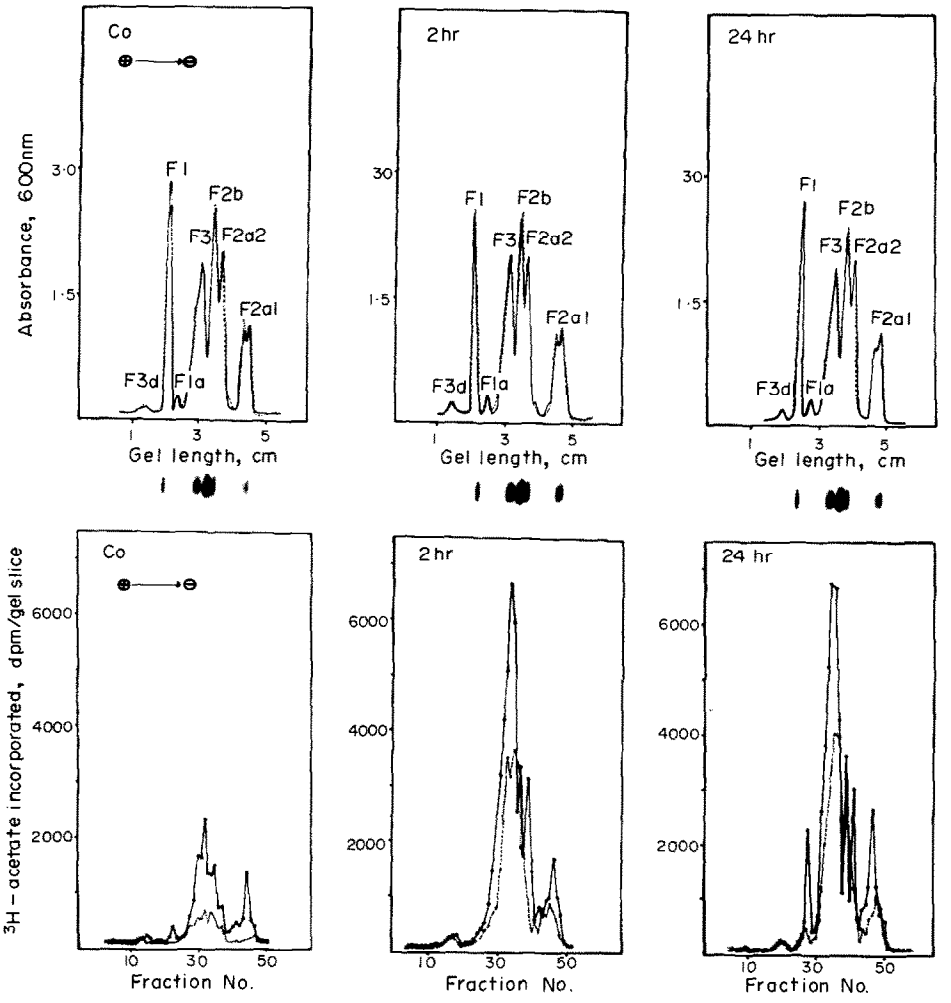

Fig. 4. *In vitro* uptake and retention of [^{3}H]acetyl groups in liver histone fractions of control rats and of rats pretreated with α-HCH for 2 and 24 hr respectively. *Upper part*: Densitometric tracings of the banding profiles of acetylated (——) and partially deacetylated (– – –) histones. The photograph shows the banding patterns of the acetylated histones which were identical with the banding patterns of the partially deacetylated histones. *Lower part*: [^{3}H]acetate uptake (——): Liver nuclei (2 mg DNA) isolated from control and α-HCH treated rats were incubated with 18·5 μCi [^{3}H]acetylcoenzyme A (sp. act. 0·92 Ci/m-mole) in 0·32 M sucrose containing 3 mM $MgCl_2$ and 20 mM Tris–HCl pH 7·4 at 37°. After a 20 min incubation period when histones were maximally labelled aliquots of the incubation assay were pipetted into ice-cold H_2SO_4 up to a final concentration of 0·4 N from which histones were prepared as described under Methods. [^{3}H]acetate retention (· · ·): At 20 min of incubation further [^{3}H]acetate uptake into histones was stopped by adding a 40-fold excess of unlabelled acetylcoenzyme A. After further incubation for 40 min the reaction was terminated by ice-cold H_2SO_4 and histones were extracted as described under Methods. 120 μg acetylated (——) or partially deacetylated (– – –) histones were applied to each gel column (0·6 × 13 cm) and electrophorised in 15% polyacrylamide containing 3·125 M urea, pH 2·8. Gels were stained, destained, scanned at 600 nm, sliced in 1 mm fractions and radioanalyzed as described under Methods.

tylated to varying degrees, the [^{3}H]acetate uptake and retention in main histone fractions separated in polyacrylamide gels were determined 2 and 24 hr after α-HCH treatment (Fig. 4).

For that purpose gel electrophoretic resolution of F3, F2b, F2a2 histones had to be improved. Resolution of stained histone bands was best achieved at 3·125 M urea and the conditions shown in Fig. 3a. Additionally arginine-rich histone fractions which are known as being the predominantly acetylated fractions [20, 21] were extracted selectively from chromatin which was depleted of F1, F2b, F2a2 histones according to Bolund and Johns [9] (Fig. 3b).

As can be seen from Fig. 4 the [^{3}H]acetate associated with the F3, F2b and F2a histone fractions of α-HCH treated and control rats accounts for most of the radioactivity taken up during 20 min of incubation. The amounts of radioactive acetate incorporated into F3, F2b and F2a fractions from rats pretreated with α-HCH for 2 and 24 hr are nearly three times higher than those found in the control fractions. Histone fraction F1 of control as well as of α-HCH

treated fractions takes up only negligible amounts of [^{3}H]acetate.

The protein fraction migrating in polyacrylamide gels between F1 and F3 histone bands (F1a according to Marsh and Fitzgerald [22]) which was defined by Panyim and Chalkley [23] as a lysine-rich histone fraction occurring only in slow replicating tissues contained several times more radioactive acetate 24 hr after α-HCH treatment than the corresponding control fraction.

The most apparent difference between the [^{3}H]acetate retention of control and treated groups refers to fraction F2a1. The acetylated F2a1 histone of control rats was depleted of nearly all of the [^{3}H]acetate incorporated at the end of the chasing period while the F2a1 fraction from rats pretreated with α-HCH for 2 and 24 hr retained considerable amounts of [^{3}H]acetate. This refers also to the F3 fraction: A considerably higher percentage of the maximum [^{3}H]acetate uptake is retained 2 and 24 hr after α-HCH application as compared to controls. This is shown in Fig. 4 and was confirmed by the results of double-labelling experiments (Fig. 5). In these experiments artefactual influences on the results possibly due to differences in isolation or degradation of histones from control and α-HCH treated rat liver nuclei are minimized. Isolated nuclei from rats treated with α-HCH for 24 hr were incubated with [^{3}H]acetylcoenzyme A and nuclei of control rats were incubated separately either with [^{3}H]acetylcoenzyme A or with [^{14}C]acetylcoenzyme A. From the ^{3}H- respectively ^{14}C-labelled liver nuclei the acetylated histone fractions F3 and F2a1 were prepared selectively as described under Methods. Equal amounts of protein of ^{14}C-labelled control histones were mixed with ^{3}H-labelled control histones or with ^{3}H-labelled histones from α-HCH treated rats and analysed electrophoretically on polyacrylamide gels. The isotopic ratio ^{3}H(dis/min)/^{14}C(dis/min) which was determined in each gel slice (Fig. 5) provides an index of differential acetylation and acetate retention in the histones of α-HCH treated and control groups. The ^{3}H/^{14}C activity ratio indicates that the rate of acetylation and acetate retention in the histones of the control groups is the same regardless of the type of labelled acetylcoenzyme A employed (Fig. 5). It can further be seen from Fig. 5 that the histone fractions F3 and F2a1 from α-HCH treated rats take up three times as much and retain nearly six times as much acetate as the corresponding control fractions.

The increased nuclear acetylation of rat liver histones at 2 and 24 hr after α-HCH application precedes and accompanies increased nuclear *in vitro* RNA synthesis [3, 4]. To approach the problem whether there is more than a temporal relationship between these two effects of α-HCH, the influence of preincubation of rat liver nuclei with acetylcoenzyme A on the nuclear *in vitro* RNA synthesis was examined. Table 1 demonstrates that the already described increase of both the nuclear RNA polymerase activities 24 hr after α-HCH application is additionally enhanced by preincubating the rat liver nuclei with acetylcoenzyme A whereas RNA polymerase activities in liver nuclei from control rats and rats pretreated with α-HCH for 2 hr did not respond to preincubation with acetylcoenzyme A (Table 1).

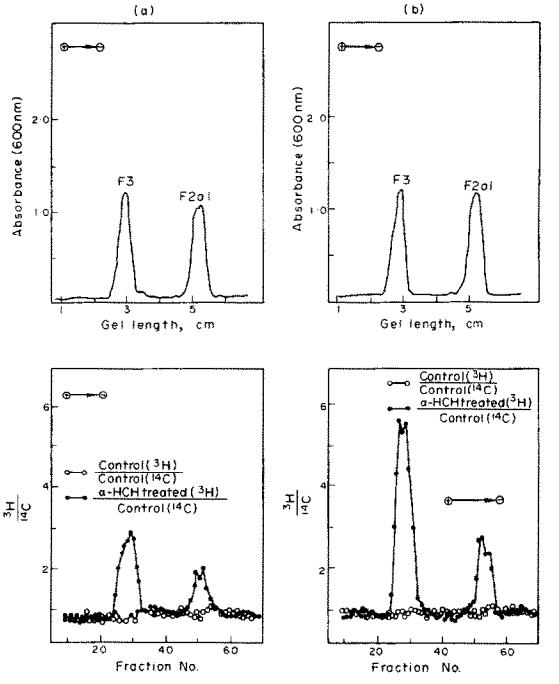

Fig. 5. *In vitro* acetylation (a) and acetate retention (b) in F3 and F2a1 liver histone fractions from control rats and rats pretreated with α-HCH for 24 hr. 30 μg of ^{14}C-acetylated F3 and F2a1 liver histones from control rats were dissolved in 0·9 N acetic acid and mixed either with equal amounts of ^{3}H-acetylated F3, F2a1 histone fractions from the same control rats or with ^{3}H-acetylated histones from α-HCH treated rats and electrophorized on polyacrylamide gels. *The upper part* (*a*) shows the optical scan of the mixture containing equal parts of ^{3}H-acetylated histone fractions F3 and F2a1 from α-HCH treated and ^{14}C-acetylated histone fractions F3 and F2a1 from control rats identical with the scan of the mixture containing equal amounts of ^{3}H-acetylated and ^{14}C-acetylated F3, F2a1 fractions from control rats. *The lower part of* (*a*) shows the ^{3}H/^{14}C ratio in each 1 mm slice derived from the polyacrylamide gels on which the mixtures of acetylated F3, F2a1 histone fractions were electrophorized. *The upper part of* (*b*) shows the optical scan of the electrophorised mixture of 30 μg partially deacetylated, i.e. [^{14}C]acetate retaining histones from control rats with 30 μg of [^{3}H]acetate-retaining histones from α-HCH treated rats identical with the optical scan of the mixture containing equal amounts of ^{3}H- and ^{14}C-acetylated F3 and F2a1 from control rats. *The lower part of* (*b*) shows the ^{3}H/^{14}C ratio in each 1 mm slice of the gels on which the mixtures of the acetate-retaining F3 and F2a1 histone fractions were electrophorised. The double-labelling procedure, the *in vitro* determination of histone acetylation and acetate retention, the isolation of the arginine-rich histone fractions F3 and F2a1, and the electrophoretic conditions are detailed under Methods.

DISCUSSION

There are several indications of qualitative and quantitative alterations of transcription of rat liver genes during the prereplicative phase of α-HCH induced rat liver proliferation. Microsomal and cytoplasmic liver enzymes show Actinomycin D sensitive increases of activity after α-HCH application [24]. Both the endogenous nuclear RNA polymerase activities increase with maximum values between 24 and

Table 1. Effect of preincubation of rat liver nuclei with acetylcoenzyme A on nuclear RNA polymerase activities

Time (hr) after administration of		Preincubation with (+) or without (−) acetylcoenzyme A	[^{3}H]UMP incorporated (dis/min per μg DNA) under conditions of	
Rape seed oil	α-HCH		Low ionic strength	High ionic strength
	2	+	28·0 ± 2·1	67·1 ± 3·7
	2	−	28·4 ± 1·9	64·4 ± 2·7
2		+	28·8 ± 1·7	68·6 ± 2·3
2		−	32·0 ± 2·9	70·0 ± 3·1
	24	+	*57·9 ± 2·9	*124·1 ± 4·5
	24	−	*46·1 ± 1·8	*96·8 ± 3·9
24		+	30·8 ± 4·0	68·6 ± 3·1
24		−	31·7 ± 2·3	72·3 ± 4·3

Liver nuclei from control rats and rats pretreated with α-HCH for 2 and 24 hr were preincubated in 0·32 M sucrose with (+) and without (−) 80 nmoles acetylcoenzyme A/mg DNA at 37°. After 5 min preincubation 0·2 ml of the nuclei suspension (about 50 μg DNA) were transferred to the RNA polymerase assay and RNA polymerase I and II activities were measured under low and high ionic strength conditions as described under Methods. Each value represents the arithmetic mean ± S.D. from three experiments. P was calculated according to Student's *t*-test. *P < 0·002.

48 hr [3, 4, 25] (Table 1). The capacity of rat liver chromatin to serve as a template for *E. coli* RNA polymerase activity was enhanced at 24 hr after α-HCH [4]. Mechanisms by which genetic restriction and structure of chromatin are changed may involve structural alterations of basic chromosomal proteins [26, 27] achieved by enzymatic side chain modifications such as histone acetylation [5, 6, 28], phosphorylation [29, 30], methylation [31], thiolation [32] etc.

Recently we reported on the temporal relationship of enzymatic histone modifications and increased RNA synthesis in rat liver after α-HCH application [3].

The [^{3}H]acetate chasing experiments of the present study clearly show that radioactive acetate originally transferred *in vitro* from acetylcoenzyme A to rat liver histones was retained in histones by nuclei isolated 2 and 24 hr after α-HCH to a greater extent than in the histones of control rats (Figs. 2 and 4). The so-determined α-HCH caused increase in acetate content represents acetyl groups bound to histone molecules already integrated in the chromatin complex because histone synthesis has been shown to occur on cytoplasmic polysomes [33, 34]. Studies on the *in vivo* labelling of rat liver histones revealed that 24 hr after α-HCH application the incorporation of radioactive amino acids increased for 20%. Assuming that a similar extent of the chromatin-bound histones is newly synthesized this would imply that most or almost all of the radioactive acetyl groups from histones isolated 24 and 2 hr after α-HCH administration are associated with preformed histone molecules. The increased retention of radioactive acetate in the α-HCH treated groups mainly refers to the histone fractions F3, F2b, F2al and is due to the several times higher uptake of the [^{3}H]acetate into these histone fractions of α-HCH treated rats but is not due to a decreased rate of [^{3}H]acetate release from these fractions. Considerably more [^{3}H]acetate was released from F3, F2b, F2al histone fractions and per μg total rat liver histone 2 and 24 hr after α-HCH application. This seems to be in contrast to experimental models where a diminished deacetylation of histones was correlated to increased RNA synthesis [6, 35]. Sanders *et al.* [35] and Pogo *et al.* [6] studied acetylation and deacetylation respectively of histones after offering radioactive acetate to whole cells or to animals, whereas we examined nuclear processes exclusively, i.e. the transfer of [^{3}H]acetyl from [^{3}H]acetylcoenzyme A to chromatin bound histones and the [^{3}H]acetate retention in these histones. For this reason no comparison is possible between their results and our findings. However, we believe that changes in amount and position of acetyl groups in chromatin bound histones are easier to correlate to chromatin-templated RNA synthesis than changes in histone acetylation or deacetylation rates.

Our recent study on the effect of histone acetylation on endogenous RNA polymerase activities in neuronal and glial rat brain nuclei [36] supports the view that the acetylation of a limited number of histone acetate binding sites is rather more important for a change in transcriptional activity than acetylation of all the histone sites open for *in vitro* acetylation.

In the α-HCH model a connection between increased transcriptional activity and increased content of nuclear acetate in rat liver histones is suggested by two of our findings. Chromatin isolated at 24 hr after α-HCH contains histone fractions with considerably more acetyl groups than the corresponding control fractions [3] and exhibits an increased template capacity for heterologous RNA polymerase [4]. Preincubation with acetylcoenzyme A of rat liver nuclei isolated 24 hr after α-HCH leads to a additional increase of their endogenous RNA polymerase activities. Two hr after α-HCH administration, the increased acetate content of histone fractions coincides neither with an increase of nuclear RNA polymerase activities nor with an enhanced template activity of chromatin [4]. Preincubation with acetylcoenzyme A of rat liver nuclei isolated 2 hr after α-HCH administration does not result in an increased nuclear RNA synthetic activity. Therefore the assumed relation between acetate content of histones and nuclear RNA synthesis can not be a simple interaction between two components. However, at present we do not know all the events involved in the regulation of chromatin-templated transcription nor their

sequence of action. Besides an increased acetate content of chromatin-bound histone fractions most likely multiple changes in the rat liver interphase nucleus, particularly in chromatin, have to occur in order to guarantee the increased transcription observed 24 hr after α-HCH administration.

Acknowledgements—The generous support of Prof. H. Herken is gratefully acknowledged. We are indebted to Mr. H. Dietz for construction of the destainer. Thanks are due to Mrs. M. Kemmerle, Miss I. Reinsch and Mrs. B. Schröder for expert technical assistance.

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