Effect of Cortisol on the Thiol Content of Rat Liver Nuclear Proteins

By D. DOENECKE, M. BEATO,* L. F. CONGOTE† and C. E. SEKERIS

*Institut für Physiologische Chemie der Universität Marburg,
355 Marburg (Lahn), Lahnberge, Germany*

(Received 30 August 1971)

Administration of cortisol to normal or adrenalectomized rats leads within 15–30 min to an increased thiol content of nuclear proteins, measured by the incorporation of iodo[^3H]-acetate or N -[^{14}C]ethylmaleimide or by colorimetric methods. The same effect is observed after incubation of isolated rat liver nuclei with corticosteroids. The increased thiol content of the nuclear proteins shows the same time-dependence as the stimulation of RNA synthesis by corticosteroids observed *in vivo* and *in vitro*. Amino acid analysis of the carboxymethylated proteins reveals that in the experiments *in vivo* most of the label is present as carboxymethylcysteine with small amounts of carboxymethyl-lysine and carboxymethylhistidine, whereas in the experiments *in vitro* more carboxymethyl-lysine and carboxymethylhistidine than carboxymethylcysteine are found. The increase in the content of thiol groups is due to cleavage of the disulphide bridges between the nuclear proteins. Polyacrylamide-gel electrophoresis of the acid-soluble fraction reveals that most of the iodo[^3H]acetate label is incorporated into a non-histone fraction with a molecular weight of approx. 45 000 whereas in the acid-insoluble fractions many protein bands are labelled.

Evidence is accumulating for the involvement of chromosomal proteins in the control of genetic expression of eukaryotic organisms. Extraction of basic protein fractions from chromatin results in an increase of its template activity for RNA synthesis (Huang & Bonner, 1962) and in a change in the nature of the RNA synthesized. The experiments of Paul & Gilmour (1968) have focused attention on the importance of non-histone chromosomal proteins for the specificity of the transcribed RNA. In our search for the initial mechanism by which cortisol increases RNA synthesis and induces specific enzymes in rat liver (Lang *et al.*, 1968) we have postulated an interaction of the hormone with chromosomal proteins (Sekeris *et al.*, 1969). This hypothesis was based on studies of the distribution of [^3H]cortisol after parenteral administration (Beato *et al.*, 1969*a*) or after incubation of isolated rat liver nuclei with cortisol *in vitro* (Beato *et al.*, 1969*b*) as well as on the effects of the hormone on isolated chromatin *in vitro* (Beato *et al.*, 1970). It was demonstrated that the increased stimulation of RNA synthesis observed after incubation of rat liver chromatin with cortisol was due not to its interaction with the RNA polymerase, nor with the DNA, but very probably with the protein moiety of the template. We have explored

further the nature of the modification of chromosomal proteins induced by cortisol. Neither acetylation nor methylation of histones was affected by incubating rat liver nuclei with cortisol under conditions leading to an increased RNA polymerase activity (Gallwitz & Sekeris, 1969). Phosphorylation of nuclear proteins after incubation of the nuclei with cortisol also was not affected (Schiltz, 1970). The only evident modification that we could demonstrate in these experiments was an increased carboxymethylation of the proteins by iodo[2- ^3H]acetate (Sekeris *et al.*, 1968). In the present paper we present evidence that this effect is due to an increase in the amount of free thiol groups of the nuclear proteins, that this increase is observed both after application of the hormone *in vivo* as well as *in vitro*, and that the increase parallels the effect of cortisol on RNA synthesis in rat liver nuclei.

Materials and Methods

Iodo[2- ^3H]acetate (sp. radioactivity 71 mCi/mmol) and iodo[2- ^{14}C]acetate (sp. radioactivity 15.5 mCi/mmol) were purchased from The Radiochemical Centre, Amersham, Bucks., U.K. N -[1- ^{14}C]ethylmaleimide (sp. radioactivity 3.63 mCi/mmol) was a product of Schwarz Bioresearch Inc., Orangeburg, N.J., U.S.A. 4,4'-Dithiopyridine (Aldrithiol) was purchased from Aldrich Chemical Co., Milwaukee, Wis., U.S.A. Steroids were kindly provided by Schering A.G., Berlin. Triton X-100 was obtained

* Present address: Columbia University, College of Physicians and Surgeons, Institute of Cancer Research, New York, N.Y., U.S.A.

† Present address: Department of Endocrinology, Royal Victoria Hospital, Montreal 112, Que., Canada.

from Serva, Heidelberg, Germany. All other chemicals were analytical grade, from Merck A.G., Darmstadt, Germany.

Male Wistar BR II rats weighing 120–130 g and kept under standard conditions were adrenalectomized 60 h before administration of cortisol. In the experiments *in vivo* doses of 0.5–5.0 mg/100 g rat were injected intraperitoneally in 0.5 ml of 0.14 M-NaCl; control rats received 0.5 ml of 0.14 M-NaCl. After different times rats were killed by cervical dislocation and liver nuclei were isolated by treatment with Triton X-100 (Beato *et al.*, 1969b). Nuclear sediment was obtained by hypo-osmotic lysis of the isolated nuclei (Beato *et al.*, 1968). The lysed nuclei were centrifuged at 8000g for 10 min and the resulting nuclear sediment was suspended in 0.065 M-tris-HCl buffer, pH 7.9. The DNA/protein ratio of the nuclear sediment was 1:4.

In the experiments *in vitro*, nuclei of intact or adrenalectomized rats were incubated in 5 ml of TSS buffer (0.25 M-sucrose–0.025 M-KCl–0.01 M-MgCl₂–0.05 M-tris-HCl, pH 7.55) containing 25 µg of cortisol in 50 µl of ethanol, the control nuclei being incubated in the same buffer containing 50 µl of ethanol. After this treatment, hypo-osmotic lysis was performed as in the experiments *in vivo*.

Protein was determined by the method of Lowry *et al.* (1951). Bovine serum albumin or calf thymus histones were used as standards.

The thiol content of nuclear proteins was measured either by the uptake of iodo[³H]acetate, iodo[¹⁴C]-acetate or *N*-[¹⁴C]ethylmaleimide, or by spectrophotometric measurement of the conversion of 4,4'-dithiopyridine into 4-thiopyridone (Grassetti & Murray, 1967). Disulphide bonds were determined by cleavage with NaBH₄ and subsequent measurement of the total thiol content of the sample.

Determination of thiol groups by the radioactivity assay was performed by incubating 100 µl of the nuclear material (containing 0.5–1.0 mg of protein) with 0.3 ml of solution of 8 M-urea in tris buffer, pH 7.9, and 0.1 ml of tris buffer (0.065 M, pH 7.9) containing 2.5 µCi of iodo[2-³H]acetate or 0.1 µCi of *N*-[1-¹⁴C]ethylmaleimide. This mixture was incubated for 30 min at 37°C, samples were pipetted on to filter-paper discs (Schleicher and Schuell, 2043b) and dropped into ice-cold 15% (w/v) trichloroacetic acid. After 20 min the paper discs were treated with 15% trichloroacetic acid at 100°C for 15 min, washed with cold trichloroacetic acid, methanol, methanol-ether (1:1, v/v) and with ether. The dried paper discs were placed in vials containing a scintillation fluid composed of 5 g of 2,5-diphenyloxazole and 200 mg of 1,4-bis-(5-phenyloxazol-2-yl)benzene/litre of toluene and counted for radioactivity in a Nuclear-Chicago Mark I liquid-scintillation spectrometer. The thiol content of the sample was determined from the measured uptake of radioactive material and the

protein content of the nuclear preparation, and expressed as c.p.m./mg of protein. With these values the hormonal stimulation was expressed as per cent above the control.

The spectrophotometric method for the determination of thiol groups was essentially that of Grassetti & Murray (1967), involving incubation of 0.2 ml of nuclear sediment with 0.5 ml of the Aldrithiol reagent, containing 22.5 mg of 4,4'-dithiopyridine/100 ml (1 mM). After 10 min of incubation the samples were centrifuged at 4500g for 5 min and the supernatant was diluted to 3 ml with tris buffer, pH 7.9. The *E*₃₃₀ was measured and compared with a calibration curve obtained with cysteine in 0.065 M-tris buffer, pH 7.9. The number of thiol groups thus determined was related to the protein content of the nuclear material.

Total thiol groups after cleavage of disulphide bonds were measured by incubating 0.2 ml of the nuclear sediment with 2.0 ml of tris buffer containing 8 M-urea and 0.1 M-NaBH₄. After 30 min at 37°C the reaction was stopped with 0.2 ml of 5 M-HCl, followed by neutralization with 0.1 ml of tris buffer. After a 10 min incubation the solution was centrifuged for 5 min at 4500g and the *E*₃₃₀ was measured.

The fractionation of nuclear proteins into acid-soluble and acid-insoluble fractions was performed after incubation of the nuclear material for 120 min together with 1.5 ml of 9 M-urea in tris buffer, pH 7.9, and 12.5 µCi of iodo[2-³H]acetate or iodo[2-¹⁴C]-acetate. Consequently, nuclear proteins were fractionated by extraction in 0.2 M-HCl for 60 min at 0°C, followed by centrifugation at 8000g for 10 min. A single extraction with acid is sufficient to remove 95% of the acid-soluble proteins (Laurence *et al.*, 1963). The acid-soluble material in the supernatant was precipitated with 10 vol. of acetone at 0°C and the proteins were collected by centrifugation for 10 min at 45000g.

Both the acid-soluble and acid-insoluble proteins were then heated in a water bath at 100°C for 15 min in 15% (w/v) trichloroacetic acid, resuspended in methanol, centrifuged again and dried. For further analysis both fractions were resuspended in the solvent required for electrophoresis or amino acid analysis.

Analytical gel electrophoresis of nuclear acid-soluble proteins was performed by the method described by Weber & Osborn (1969). The determination of the molecular weight was performed by the method of Weber & Osborn (1969) with cytochrome c, chymotrypsinogen, ovalbumin, bovine serum albumin and catalase as standards. In all experiments the Shandon equipment was used. For preparative polyacrylamide-gel electrophoresis the Canalco apparatus was used. The procedure described by McAllister *et al.* (1963) for analytical electrophoresis was used.

For amino acid analysis either direct hydrolysis

of the dried protein fraction, prepared as described above, was performed, or the paper discs used in the assay for incorporation of radioactive material were first eluted with 0.1 M-NaOH for 60h at 50°C and the eluates dried in a flash evaporator and hydrolysed by incubation with 6M-HCl in evacuated glass tubes at 105°C for 20h. The hydrolysate was dried again in a flash evaporator and dissolved in 0.2M-sodium citrate-HCl buffer, pH2.2. Amino acid analysis was then performed by standard procedures with a Beckman Unichrom amino acid analyser.

The assay for RNA polymerase activity was essentially the same as described by Lukacs & Sekeris (1967). Under these conditions both amanitin-sensitive and amanitin-insensitive DNA-dependent RNA polymerases are assayed (W. Schmid & C. E. Sekeris, unpublished work).

Results

Effects of cortisol in vivo on the thiol content of nuclear proteins and on nuclear RNA polymerase activity

Fig. 1 summarizes the effect of intraperitoneal administration of cortisol on the incorporation of iodo[2-³H]acetate into the nuclear proteins and on the RNA polymerase activity of rat liver nuclei. The

Fig. 1. *Effect in vivo of cortisol on the incorporation of labelled iodoacetate into rat liver nuclear sediment and on RNA polymerase activity of the nuclear sediment*

Rats weighing 125 g were killed 30min after intraperitoneal administration of the hormone. RNA polymerase activity and thiol content were determined as described in the text. The measured uptake of radioactive material was expressed as c.p.m./mg of protein and the hormonal stimulation calculated as a percentage of the control (broken line). Δ , RNA polymerase activity; $\circ$, incorporation of iodo[³H]-acetate.

optimum dose of cortisol was the same for the stimulation of both parameters, i.e. 2mg/100g body wt., roughly corresponding to the dose required for the induction of hepatic tyrosine transaminase (Kenney & Kull, 1963). The amplitude of both cortisol effects was similar when the average value was considered.

The time-dependence of the cortisol stimulation of both the incorporation of iodo[2-³H]acetate and of the RNA polymerase activity in the liver nuclei from intact rats is shown in Fig. 2. At the range of cortisol doses tested the hormonal effect on the incorporation of iodo[2-³H]acetate and on RNA polymerase activity was maximal 30min after administration of cortisol. In intact animals the incorporation of iodo[2-³H]acetate into nuclear proteins and RNA polymerase was slightly inhibited 120min after administration of cortisol. When adrenalectomized animals were used, the magnitude of the cortisol effect on incorporation of ³H was similar to that observed in intact animals, but the stimulation persists for up to 2h after injection of the hormone.

The increased incorporation of iodo[2-³H]acetate into nuclear proteins of cortisol-treated animals is due to the reductive cleavage of disulphide bridges. This has been shown by measuring the ratio of thiol to thiol plus disulphide groups of the nuclear proteins, as determined by the dithiopyridine reagent (Grassetti & Murray, 1967). The ratio shows a significant increase 30min after injection of cortisol (Table 1a). The values for control and cortisol-treated

Fig. 2. *Time-dependence of the effect of cortisol on the incorporation of labelled iodoacetate and on RNA polymerase activity*

Percentage change in incorporation of labelled iodoacetate into nuclear proteins, from intact ($\circ$) and adrenalectomized ($\bullet$) rats, and in RNA polymerase activity (Δ) of the nuclear sediment after intraperitoneal injection of cortisol are shown. For experimental conditions and calculation of results see the text. The broken line indicates the control value.

Table 1. *Effect of cortisol on the thiol content of rat liver nuclear proteins*

For experimental details see the text.

(a) Ratio of thiol to thiol plus disulphide groups (determined by the dithio-pyridine reagent).

	Thiol content (nmol/mg of nuclear protein)	
	Control animals	Cortisol-treated animals
Before NaBH ₄ treatment	0.280	0.500
After NaBH ₄ treatment	0.447	0.560
Ratio (-SH)/(-SH)+(S-S)	0.628	0.895

(b) Titration of nuclear protein preparations from control and cortisol-treated rats with labelled iodoacetate.

	Iodo[³ H]acetate incorporated into nuclear sediment (c.p.m./mg of protein)		Percentage effect
	Control nuclei	Cortisol-treated nuclei	
Complete system	29450	32950	+12
-8M-Urea	11820	13225	+12
+3.5M-NaCl	62050	67850	+ 9
+0.1% Sodium dodecyl sulphate	26810	29600	+11
+Sodium thioglycollate	91020	91800	+ 1
+Sodium borohydride	86100	87910	+ 2

Fig. 3. *Amino acid analysis of the labelled nuclear proteins prepared from the rat liver nuclear sediment from control and cortisol-treated animals*For the preparation of labelled protein hydrolysate see the text. The same amount of protein hydrolysate from control (Δ) and cortisol treated ($\circ$) animals was analysed., E_{570} .

nuclei roughly agree with those reported for hetero- and eu-chromatin respectively (Ord & Stocken, 1966). Similarly, if the nuclear protein preparations from control and cortisol-treated animals are titrated with labelled iodoacetate after cleavage of the disulphide bridges with sodium thioglycollate or sodium borohydride total incorporation of iodoacetate is increased whereas the difference between control and cortisol-treated preparations disappears. The stimulatory effect of cortisol is still apparent in protein preparations treated with 3.5 M-NaCl or 0.1 % sodium dodecyl sulphate and also in the absence of urea (Table 1b).

Amino acid analysis of the labelled nuclear proteins prepared from the liver of control and cortisol-treated animals shows that most of the label is recovered in the form of carboxymethylcysteine and that the amount of radioactivity in this region of the chromatogram is significantly greater after treatment with cortisol (Fig. 3). When the proteins from nuclei labelled

with iodo[2-³H]acetate are separated by extraction with 0.2 M-HCl (or 0.25 M-H₂SO₄) the specific radioactivity of the acid-insoluble proteins is 1.5-fold that of the acid-soluble fraction. However, the cortisol effect on the incorporation of iodoacetate was observed in both fractions to be approximately of the same value.

Electrophoresis of the labelled acid-soluble nuclear proteins in polyacrylamide gels demonstrates that the major fraction of the label does not move with any of the main histone fractions. In the presence of 0.1 % sodium dodecyl sulphate, where the proteins are separated exclusively on the basis of molecular-weight differences, a radioactivity peak is observed in a region corresponding to a molecular weight between 40 000 and 50 000, much higher than that of the histone fractions (Fig. 4).

Electrophoretic separation of the acid-insoluble nuclear proteins after administration of cortisol *in vivo* was performed at pH 7.0 in the presence of 0.1 % sodium dodecyl sulphate (Weber & Osborn, 1969). Under these conditions no clearcut qualitative differences between the electrophoretic distribution of the labelled acid-insoluble proteins from control and cortisol-treated animals could be observed in double-labelling experiments.

Increased thiol content of proteins of rat liver nuclei incubated with cortisol

Incubation of isolated rat liver nuclei with cortisol results in an increased incorporation of labelled iodoacetate into the nuclear proteins (Sekeris *et al.*, 1968).

Fig. 4. Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate of the labelled acid-soluble rat liver nuclear proteins

Labelled acid-soluble rat liver nuclear proteins of control (●) and cortisol-injected (□) animals were subjected to electrophoresis. The ciphers in the graph refer to histone fractions.

Fig. 5. Effect of cortisol phosphate on the incorporation of labelled iodoacetate into nuclear proteins *in vitro*

Nuclei were incubated for 10 min at 37°C in 5 ml of buffer containing the concentrations of cortisol phosphate indicated. The measured uptake of labelled iodoacetate was expressed as c.p.m./mg of protein and the hormonal effect expressed as a percentage of the control.

Fig. 5 shows the dose-dependence of this effect, which parallels the hormone concentrations required for the stimulation of RNA polymerase activity in isolated nuclei. At optimum concentrations of cortisol a 15% effect could be detected, which was statistically significant ($P < 0.01$ for 21 experiments) and could be observed after 10 min of incubation at 37°C. As in the experiments *in vivo*, determination of the ratio of thiol to thiol plus disulphide groups in the nuclear proteins of control nuclei and nuclei incubated with cortisol demonstrates that the hormone causes the cleavage of disulphide bridges. In agreement with this observation, the stimulation by cortisol of the incorporation of iodoacetate is also evident in the presence of urea or NaCl and disappears, as in the experiments *in vivo*, after treatment with NaBH_4 .

Extraction of the labelled nuclei with 0.2M-HCl shows that both the acid-soluble and acid-insoluble nuclear proteins are involved in the response to cortisol. When the acid-soluble proteins are separated by preparative polyacrylamide-gel electrophoresis, results very similar to those reported for the experiments *in vivo* are obtained (Fig. 6). After incubation of the nuclei with cortisol the main fraction of the labelled protein also moves more slowly to the cathode than do the main histone fractions. Similar patterns were observed in analytical gel electrophoresis.

The acidic proteins from chromatin prepared

according to Marushige & Bonner (1966) were also analysed after labelling of the nuclei with ^{14}C - or ^3H -labelled iodoacetate. After incubation of the isolated nuclei with cortisol chromatin was prepared and the acidic proteins were purified by extraction with 2.5M-NaCl–5M-urea and filtration through Bio-Rex A20 (Richter & Sekeris, 1972). When this fraction was applied to a DEAE-Sephadex column, washed and eluted with a NaCl gradient, a complex but reproducible pattern of protein peaks was observed. However, no significant difference between control and hormone-treated preparations was observed.

Amino acid analysis of the acid-soluble as well as the acid-insoluble fraction shows not only the presence of carboxymethylcysteine but also of carboxymethyl-lysine and carboxymethylhistidine (Figs. 7a and 7b). These last two derivatives were not found in the carboxymethylation of nuclear proteins from rats stimulated *in vivo*. After administration of cortisol the relative and absolute amount of carboxymethylcysteine increased considerably over the controls.

Effect of actinomycin D on the thiol content of nuclear proteins

Table 2 summarizes the effect of actinomycin D on the thiol content of nuclear proteins of control and

Fig. 6. *Preparative polyacrylamide-gel electrophoresis of acid-soluble rat liver nuclear proteins*

Results were obtained after incubation of the proteins with iodo[^{14}C]acetate (control nuclei, Δ) or iodo[^3H]acetate (cortisol-treated nuclei, $\circ$). , E_{405} .

Fig. 7. Amino acid analysis of (a) the acid-soluble and (b) the acid-insoluble rat liver nuclear proteins

Proteins were from control nuclei (Δ) and nuclei treated *in vitro* with cortisol ($\circ$). $\cdots\cdots$, E_{570} . The same amount of protein hydrolysate was analysed; for experimental details see the text.

cortisol-stimulated nuclei. Although actinomycin D in the dose used decreases RNA synthesis to 30% of the control and abolishes the hormonal stimulation of RNA synthesis it has no effect on the increased thiol content of the nuclear proteins.

Discussion

The molecular mechanism of the activation of the genetic material has been the object of considerable attention during recent years. One approach has been to follow changes in the enzymic modification of the

Table 2. *Effect of actinomycin D on the thiol content of nuclear proteins from control and cortisol-treated rat liver nuclei compared with its influence on RNA polymerase activity*

For experimental details see the text. Rat liver nuclei were incubated for 10 min at 37°C.

Addition to incubation mixture ...	Cortisol (5 µg/ml)	Actinomycin D (20 µg/ml)	Cortisol + actinomycin D (5 µg + 20 µg/ml)
Incorporation of iodoacetate (% of control)	118.5	84.5	128.0
RNA polymerase activity (% of control)	123.1	38.0	31.0

histones by acetylation, phosphorylation and methylation and to try to correlate them with the increased template activity of chromatin. Another line of approach was to follow possible conformational changes of nuclear proteins evoked by a direct interaction with the hormone and connected to the increased transcription. In a first series of experiments we have measured the thiol content of nuclear proteins as a way to detect changes in protein conformation. We have detected a very rapid increase in the thiol content of nuclear proteins after the action of corticosteroids either *in vivo* or *in vitro*, as measured either by the incorporation of iodo[2-³H]acetate or by colorimetric methods. That the incorporation of iodoacetate is a measure of the thiol groups was checked by amino acid analysis of the nuclear proteins. In the experiments *in vivo* almost all of the label was found as carboxymethylcysteine. However, in the experiments *in vitro* more carboxymethyllysine and carboxymethylhistidine than carboxymethylcysteine were found. This difference could be due to the re-formation of the disulphide bridges during the aerobic incubation of the isolated nuclei with the hormone. The loosening of the nucleoprotein structure during the incubation, rendering many masked amino or histidine groups accessible to iodoacetate titration, could also be considered. However, the increased iodoacetate incorporation elicited by cortisol in these cases is also due, as shown by the amino acid analysis, to the increase in the amount of carboxymethylcysteine present whereas the amount of carboxymethyl-lysine and carboxymethylhistidine is decreased.

The increase in thiol groups is not a consequence of the stimulation of RNA synthesis, for it is observed also in the presence of actinomycin D inhibiting RNA synthesis by more than 70%. Both acid-soluble and acid-insoluble fractions of nuclear proteins are affected. However, analysis of the acid-soluble fraction by polyacrylamide-gel electrophoresis reveals that the main protein fraction involved in the hormonal effect is not a histone but a protein of molecular weight of approx. 45000 with a mobility completely different from that of the main histone

fractions. Polyacrylamide-gel electrophoresis of the acid-insoluble proteins reveals the presence of many iodo[2-³H]acetate-labelled fractions. However, we have not been able to show conclusively the specific involvement of any of the proteins in the hormonal response, but only a general increase in the thiol content of several fractions.

The increased thiol content is not only due to a conformational change of the proteins as originally assumed, but also to a cleavage of disulphide bridges as shown by the effects of high salt concentration, urea treatment and by treatment with sodium borohydride respectively. Stocken & Ord (1966) were the first to show the existence of disulphide bridges between the nuclear proteins. Stevely & Stocken (1966) have shown that the reductive cleavage of the histone F3 dimer decreases the inhibitory action of the molecule on RNA synthesis *in vitro*. It has also been shown that after hepatectomy the ratio of thiol to thiol plus disulphide increases and that this increase parallels the increased RNA synthesis observed in regenerating liver (Ord & Stocken, 1968).

Similar changes in the thiol content of nuclear proteins of target tissues after action of other hormones have been reported recently. Congote *et al.* (1971) have found an increased thiol content of rat kidney nuclear proteins 30 min after the injection of aldosterone but no effect after oestradiol application as control. A decrease in the thiol content of proteins of isolated thymus nuclei has been observed on incubation in the presence of corticosteroids or testosterone, hormones that under the same experimental conditions inhibit RNA synthesis (Abraham & Sekeris, 1971). Radioautography has shown that the 'puff regions' of *Rhynchosciara angela* show increased grain content after incubation with iodo[2-³H]acetate (G. Weirich & C. E. Sekeris, unpublished work). As is well established, these regions of the chromosome represent the active gene sites (Beermann, 1952).

It is still premature to interpret, in molecular terms, the observed correlation between changes in the thiol/disulphide content of the nuclear proteins and functional status of the organism. This will be

possible only when these proteins have been isolated, amply characterized and their effects on transcription tested *in vitro*.

We thank Professor P. Karlson for his continued interest in our work and Mrs. C. Pfeiffer and Miss G. Fröhlich for valuable technical assistance. This work has been generously supported by the Deutsche Forschungsgemeinschaft.

References

- Abraham, A. & Sekeris, C. E. (1971) *Biochim. Biophys. Acta* **247**, 562
- Beato, M., Homoki, J., Lukacs, I. & Sekeris, C. E. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 1099
- Beato, M., Biesewig, D., Braendle, W. & Sekeris, C. E. (1969a) *Biochim. Biophys. Acta* **192**, 494
- Beato, M., Homoki, J. & Sekeris, C. E. (1969b) *Exp. Cell Res.* **55**, 107
- Beato, M., Seifart, K. H. & Sekeris, C. E. (1970) *Arch. Biochem. Biophys.* **138**, 272
- Beermann, W. (1952) *Chromosoma* **5**, 139
- Congote, L. F., Cheah, A. M. & Trachewsky, D. (1971) *Abstr. Proc. Can. Fed. Biol. Sci.* **14**, no. 234
- Gallwitz, D. & Sekeris, C. E. (1969) *FEBS Lett.* **3**, 99
- Grassetti, D. R. & Murray, J. F. (1967) *Arch. Biochem. Biophys.* **119**, 41
- Huang, R. C. & Bonner, J. (1962) *Proc. Nat. Acad. Sci. U.S.* **48**, 1216
- Kenney, F. T. & Kull, F. J. (1963) *Proc. Nat. Acad. Sci. U.S.* **50**, 493
- Lang, N., Herrlich, P. & Sekeris, C. E. (1968) *Acta Endocrinol. (Copenhagen)* **57**, 33
- Laurence, D. J. R., Simson, P. & Butler, J. A. V. (1963) *Biochem. J.* **87**, 200
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265
- Lukacs, I. & Sekeris, C. E. (1967) *Biochim. Biophys. Acta* **134**, 85
- Marushige, D. & Bonner, J. (1966) *J. Mol. Biol.* **15**, 160
- McAllister, H. C., Jr., Wan, Y. C. & Irvin, L. J. (1963) *Anal. Biochem.* **5**, 321
- Ord, M. G. & Stocken, L. A. (1966) *Biochem. J.* **98**, 888
- Ord, M. G. & Stocken, L. A. (1968) *Biochem. J.* **107**, 403
- Paul, J. & Gilmour, R. S. (1968) *J. Mol. Biol.* **34**, 305
- Richter, K. H. & Sekeris, C. E. (1972) *Arch. Biochem. Biophys.* in the press
- Schiltz, E. (1970) Doctoral Thesis, Philipps University, Marburg/Lahn
- Sekeris, C. E., Beato, M., Homoki, J. & Congote, L. F. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 857
- Sekeris, C. E., Beato, M. & Seifart, K. H. (1969) *Proc. Int. Congr. Pharmacol.* **4th**, 141
- Stevely, W. S. & Stocken, L. A. (1966) *Biochem. J.* **100**, 20c
- Stocken, L. A. & Ord, M. G. (1966) in *The Cell Nucleus—Metabolism and Radiosensitivity* (Klouwens, H. M., ed.), p. 141, Taylor and Francis Ltd., London
- Weber, K. & Osborn, M. (1969) *J. Biol. Chem.* **244**, 4406