either allyl alcohol or bromobenzene, chemically reactive metabolites may produce hepatic necrosis by alkylating tissue macromolecules, and the distribution of the binding and necrosis appears to be determined by the localization within the liver lobule of the enzymes which produce the toxic metabolites. These studies may be relevant to the mechanisms by which therapeutic agents occasionally produce liver necrosis and other tissue lesions in man.

[32] I thank Mr. JOHN GEORGE, Miss KATHY LALUSH and Mrs. MARY ALICE LARSON for their expert technical assistance.

Résumé. Ces recherches indiquent que la répartition des lésions nécrotiques produites chimiquement peut être déterminée par la localisation intrahépatique d'enzymes métabolisant les drogues, enzymes qui synthétisent, dans les cellules où ils sont produits, les métabolites chimiquement actifs capables d'alkylation des macromolécules.

W. D. REID[32]

Laboratory of Chemical Pharmacology, National Heart and Lung Institute, Bethesda (Maryland 20014, USA), 1 March 1972.

Protein Synthesis Stimulation in Rat Liver by Chloroquine

Chloroquine[1], a powerful antimalarial drug, produces a large number of biological effects, which reveals a diversity of activities. Evidence has been obtained that CQ acts on several cell sites, by binding to RNA, DNA and proteins[2–4] and by inhibiting many hydrolytic and respiratory enzymes[5]. As a consequence of its large spectrum of interactions, an inhibition on cell replication and macromolecular biosynthesis has been reported in bacterial and tissue cultures[6,7]. That CQ behaves as a cytostatic agent has been confirmed in our experiments on Yoshida ascite cells, but we have also observed that, soon after CQ injection into normal rats, the incorporation of amino acids into liver proteins is enhanced.

In fact, 4 h after an i.p. injection of 30 mg/kg body wt. of CQ in male albino Wistar rats (weighing about 150 g) the in vivo incorporation of leucine ^{14}C into liver proteins increases about 40% compared with controls. This stimulation is also observed in the liver post-mitochondrial fractions tested in a cell-free assay system.

A close dose-response relationship is present in a concentration range of 15–45 mg/kg body wt.; doses above 60 mg give frequent rise to toxic accidents. In time experiments this stimulation is appreciated within 60 min after the injection of the drug; it reaches its maximum effect after 4 h, and thereafter it slightly decreases.

In separate experiments we observed that both cytosol and pH 5-fraction prepared from CQ-treated rats are not able to stimulate the amino acid incorporation in the in vitro assay. Polysomes, however, are shown to be more active than those of the controls when tested for their endogenous mRNA (Table I). Polyuridylic acid-directed polyphenylalanine synthesis was found to be lower in polysomes from treated rats and the ratio of the incorporation values in absence and in presence of artificial messenger demonstrates that less endogenous mRNA-free sites are available in CQ-polysomes. These results also correspond to the zonal sedimentation profiles, which show a larger fraction of heavier aggregates in CQ-treated rat liver polysomes.

[1] Expressed as CQ was purchased from Wintrop Lab.
[2] K. H. MUENCH, Cold Spring Harb. Symp. quant. Biol. *31*, 539 (1966).
[3] S. COHEN and K. YIELDING, Arthritis Rheum. *7*, 302 (1964).
[4] F. S. PARKER and J. L. IRVIN, J. biol. Chem. *199*, 897 (1952).
[5] R. FIDDICK and H. HEATH, Nature, Lond. *213*, 628 (1967).
[6] G. L. LANCZ, L. C. MCLAREN, C. G. JAMES and J. V. SCALETTI, Proc. Soc. exp. biol. Med. *136*, 1289 (1971).
[7] L. HOPKINSON and F. L. JACKSON, Nature, Lond. *202*, 27 (1964).

Table I. Incorporation of ^{14}C phenylalanine into proteins by the postmitochondrial fractions and polysomes isolated from rat liver at various time intervals after a single i.p. injection of 30 mg/kg body wt. of CQ

	Postmitochondrial supernatant		Polysomes		
Time after injection	Counts per min / mg of proteins		Counts per min / mg of RNA		Incorporation ratio
			− poly U	+ poly U	+ poly U / − poly U
Control (h)	2,380	100	26,150	58,150	2.24
1	2,860	120	68,200	83,400	1.23
4	3,420	144	113,750	121,800	1.07
18	3,160	134	98,600	114,300	1.16
24	2,730	114	81,000	92,500	1.14

The complete system contained in 1 ml:
Tris-HCl pH 7.6 50 μmole; $MgCl_2$ 5 μmole; KCl 25 μmole; β-mercaptoethanol 5 μmole; ATP 1 μmole; GTP 0.4 μmole; phosphoenolpyruvate 10 μmole; phosphoenolpyruvatekinase 10 μg; aminoacids ^{12}C 6.10^{-2} μmole each; ^{14}C phenylalanine (457 mCi/mmole) 1,6 μCi; cell sap 330 μl; polysomes 10 O.D.; polyuridylic acid 1000 μg. In the experiments performed with the postmitochondrial supernatant the cell sap was omitted and 650 μl of the supernatant were used (total proteins 3 mg). After incubation for 30 min at 37 °C, aliquots were absorbed onto paper disks and processed according to MANS and NOVELLI[12].

Table II. In vivo incorporation of orotate in rat liver nuclear RNA following an i.p. injection of 30 mg/kg body wt. of CQ.

Animal[a]	Specific activity dpm/100 mg RNA	%
Control (2)	133.300 ± 8.700[b]	100
Treated (2)	129.750 ± 12.250[b]	97

100 μCi of orotate ^3H (17 Ci/mmole) was injected i.v. 4 h after drug. The animals were sacrified 20 min later. RNA was extracted from purified nuclei and the samples were processed for counting according FLOYD, OKAMURA and BUSCH[13]. [a] Number of animals in parentheses. [b] ± Mean deviation.

Table III. Incorporation capacity of liver postmitochondrial fraction of CQ-treated rats after a bilateral adrenalectomy[a]

Dose CQ mg/kg body wt.	Time (h after CQ-treatment)	Incorporation (% relative to adrenalectomized controls)
15 (4)	24	110
30 (2)	4	115

[a] Adrenalectomy was performed 1 week prior to treatment. The incorporating assay was carried out as described in Table I. Number of animals in parenthesis.

This stimulation cannot be ascribed to the interaction of the drug with the protein synthesis components, because CQ, when it is added to a cell-free assay system, produces a definite inhibition.

In order to explore the possibility that CQ mediates the increase of protein synthesis by increasing the rate of mRNA transcription, the labelling of nuclear RNA was determined. As shown in Table II, no difference was observed in the amount of the incorporated uridine between the treated and control animals. This result leads, therefore, to the assumption that the stimulation by CQ of protein biosynthesis in rat liver occurs at the post-transcriptional level.

Several experimental conditions, such as drugs, ionizing radiations, and irritative stimuli, are able to enhance the incorporation of amino acids into rat liver proteins. All these conditions, however, operate in an indirect way, by means of a stimulation of the secretory activity of the adrenal cortex, as demonstrated by the failure of such effects in the adrenalectomized animals.

Drugs, in particular, show a biphasic effect. Initially they produce an inhibition owing to their toxicity, afterwards the liver protein biosynthesis increases because of the higher level of glucocorticoid hormones induced by drugs. With CQ, however, this effect appears within 30 min after the injection and it is not preceded by an inhibition. We also observed that the incorporating ability of the postmitochondrial supernatants from rats subjected to a bilateral adrenalectomy is not depressed within the first 24 h after CQ treatment and a stimulation is still appreciated even at lower dosage of the drug (Table III). On the basis of these results it is conceivable that CQ or its metabolite(s) acts directly on liver hepatocyte rather than promoting an indirect response mediated by the increase of the glucocorticoids.

It has been shown that CQ is selectively concentrated into the liver lysosomes[8,9] and that its presence stabilizes the lysosomal membranes and retards the release of the enzymes from the lysosomes[10]. With regard to the effect of CQ on liver protein biosynthesis, it must be recalled that the lysosome may be involved in the regulation of this process by controlling the release or the activities of its nucleolytic and proteolytic enzymes, condition that is regarded as affecting the rate of mRNA degradation. This action on the permeability of the lysosomal membrane, which has been demonstrated for CQ as well as for the glucocorticoid hormones[11], may therefore play a role in the regulatory mechanism of protein biosynthesis.

Beside this property, both CQ and glucocorticoids display some anti-inflammatory and anti-rheumatic activities and both are able to stimulate the protein biosynthesis in rat liver. While this relationship is conceivably fortuitous, the possibility is suggested that their biological affinities are the expression of a convergent action upon the same target.

Riassunto. L'inoculazione endoperitoneale di Clorochina, a dosi comprese tra 15 e 45 mg/kg di peso corporeo determina una stimolazione precoce delle sintesi proteiche nel fegato di ratto. Questa azione non é correlata ad un aumento della sintesi di RNA, né appare conseguente ad una stimolazione corticosurrenalica. L'effetto osservato é brevemente discusso sulla base delle note interazioni tra lisosomi e Clorochina.

A. DELPINO and U. FERRINI

Institute Regina Elena for Cancer Research, 291 Viale Regina Elena, I-00161 Roma (Italy), 4 April 1972.

[8] R. ABRAHAM, L. GOLDBERG and P. GRASSO, Nature, Lond. *215*, 194 (1967).
[9] R. ABRAHAM, W. DAWSON, P. GRASSO and L. GOLDBERG, Expl molec. Path. *8*, 370 (1968).
[10] G. WEISSMANN, Arthritis Rheum., *9*, 834 (1966).
[11] G. WEISSMANN and H. L. THOMAS, Recent Prog. Horm. Res. *20*, 215 (1964).
[12] R. I. MANS and G. D. NOVELLI, Arch. Biochem. Biophys. *94*, 48 (1961).
[13] L. FLOYD, N. OKAMURA and H. BUSCH, Biochim. biophys. Acta 129, 68 (1966).

Fate of Internal Doses of DDT

It has been suggested[1-3] that the insect haemolymph doesn't play a significant role in the transport of topical doses of chlorinated hydrocarbon insecticides to their site of action. The present investigation deals with the role of insect circulation in the transport of injected doses of DDT.

WHO susceptible strain of houseflies was used. LC_{50} and LC_{90} for males averaging 16 mg in weight were 1 and 2.5 μg of technical DDT, respectively. 2.5 μg of a mixture of C^{14} and carrier DDT (activity 700 ± 100 cps) in 0.1 μl acetone were injected deep into the thorax between the