intact viral particles, may replicate and induce a one-cycle production when injected into such embryonated eggs. In this system also, the combination of MA and V-RNA showed to be non-infectious over a 24 h period. If combinations of V-RNA with MA remain unaltered intracellularly, then one should expect to be able to block multiplication of viruses by giving MA to HeLa cells before infection with viral particles and their nucleic acids. Experiments carried out with vaccinia (virus containing V-DNA) and poliovirus or its V-RNA component, however, were unsuccessful, because a reduced yield of particles per cell was observed but conditions necessary to shun viral replication completely have not yet been met. Toxicity of MA for HeLa makes it difficult to saturate cells with basic protein in such way that single nucleic acid molecules could be conveniently trapped before they can transfer their genetic message to the cell.

Discussion. (a) Since we have previously found[7] LMW-RNA in both the washed pellet and the supernatant of a 105000 g centrifugation, our LMW-RNA chromatography fraction is not exactly the same as transfer ribonucleic acid (S-RNA) isolated by ultracentrifugation of cellular homogenates; the same distinction must be made for HMW-RNA and R-RNA from ribosomes. Our previous[11] and present data indicate that both these ribonucleic acids, as well as cellular DNA, when combined with MA, are stable toward the hydrolytic action of nucleases, *in vitro* and *in vivo*. Such a resistance seems due to a real combination and not merely to inhibition by MA of enzyme activity, because no competition was observed in the present of an excess of ribonuclease[11].

(b) The observed uptake of naked DNA by HeLa cells confirms the reports from several Laboratories[12–16] which have recently described the penetration of homologous and heterologous DNA molecules into the nuclei of different mammalian cells.

(c) It is known that infectious centers are reduced by a factor of 3 to 5 logs upon extraction of RNA-viruses with phenol. In the case of tobacco mosaic virus, however, efficiency of infection was increased thousand fold by combining V-RNA with its protein partner[17]. Our results seem to indicate that two main factors are involved in the efficiency of infection with V-RNA: protection from extracellular nucleases and absorption on the host as well. The increased uptake of protein-bound nucleic acids that we have observed is in agreement with recent reports of similar enhancements observed for DNA, R-RNA and V-RNA combined with gelatin[18], protamin[19] and calfthymus histones[20].

(d) The system we have studied could be used as a model for investigating the action of interferon(s), which are described as protein(s) of low molecular weight synthesized by cells treated with inactivated viruses and able to inhibit multiplication of other alive challenge viral species. One hypothesis that we had postulated is that interferon(s), like our methylated albumin, may merely undergo combination with nucleic acids in a complex stable to intracellular enzymes. Further work is necessary to clarify this point.

Résumé. Les acides nucléiques se combinent avec l'albumine méthylée et forment des complexes qui ne sont pas hydrolysés par les nucléases, mais dissociés par des ions inorganiques. Ces nucléoprotéines pénètrent dans les cellules en quantité beaucoup plus élevée que les acides nucléiques correspondants, et contrairement à ceux-ci, elles ne sont pas catabolisées. La combinaison avec l'albumine méthylée rend inactif l'acide ribonucléique viral: les ions inorganiques, et non les cellules vivantes, sont capables de dissocier ce complexe et de libérer l'ARN infectieux.

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[12] S. M. GARTLER, Nature *184*, 1505 (1959).
[13] F. M. SIROTNAK and D. J. HUTCHINSON, Biochim. biophys. Acta *36*, 246 (1959).
[14] E. BORENFREUND and A. BENDICH, J. biophys. biochem. Cytol *9*, 81 (1961).
[15] M. HILL, Nature *189*, 916 (1961).
[16] R. I. SALGANIK, T. M. MOROSOVA, and V. F. DREVICH, Proc. Nat. Acad. Sci. USSR *26*, 399 (1961).
[17] H. FRAENKEL-CONRAT and B. SINGER, Biochim. biophys. Acta *24*, 540 (1957).
[18] K. G. BENSCH and D. W. KING, Science *133*, 381 (1961).
[19] H. AMOS, Biochem. biophys. Res. Comm. *5*, 1 (1961).
[20] C. E. SMULL, M. F. MALLETTE, and E. H. LUDWIG, Biochem. biophys. Res. Comm. *5*, 247 (1961).

Influence of Chlorpromazine on Nuclear and Cytoplasmic Uptake of ^{35}S-Methionine[1]

Introduction. The most evident effects of chlorpromazine on cell metabolism seem to be an increase in the ATP content of certain tissues and a derangement of the electron transfer process[2–5]. In several instances morphological degenerative changes have been observed[6,7].

The results of the influence of chlorpromazine on protein synthesis are in conflict. MITINA[8] after studying several groups of rats injected with increasing doses of chlorpromazine, up to 20 mg/kg of body weight per day for 13 days, found no difference in the ^{35}S-methionine uptake by proteins in the experimental animals, as compared with controls. LINDAN et al.[9] observed *in vitro* an inhibition of the rate of glycine-1-^{14}C incorporation into rat brain cortex proteins, in a concentration of the drug that has little or no effect on the respiration of the brain or on the rate of breakdown of glycine-1-^{14}C into $^{14}CO_2$. ZÖLLER et al.[10] found a decrease in the incorporation of glycine-^{14}C and lysine-E-^{14}C following the injection of only 5 mg/kg body weight of chlorpromazine into rats.

[1] This investigation was supported by grants from the Rockefeller Foundation and Conselho Nacional de Pesquisas.
[2] L. G. ABOOD and L. ROMANCHEK, Ann. N.Y. Acad. Sci. *66*, 812 (1957).
[3] R. G. GRENELL, Ann. N.Y. Acad. Sci. *66*, 826 (1956–1957).
[4] Y. TSUJIMURA, J. Nara Med. Ass. *7*, 25 (1957).
[5] R. G. GRENNELL, L. MAY, W. D. MCELROY, and J. MENDELSON, Res. Publ. Ass. Nerv. Ment. Dis. *37*, 417 (1959).
[6] L. ROISIN, C. TRUE, and M. KNIGHT, Res. Publ. Ass. Nerv. Ment. Dis. *37*, 285 (1959).
[7] M. HORMIA, A. HORMIA, and P. HAKOLA, Ann. Med. exp. Biol. Fenn. *35*, 316 (1957).
[8] L. V. MITINA, Pharmacology and Toxicology *20*, 75 (1957).
[9] O. LINDAN, J. H. QUASTEL, and S. SVED, Can. J. Biochem. Physiol. *35*, 1145 (1957).
[10] E. ZÖLLER, K. SCHREIER, and P. R. YANG, Arzneim.-Forsch. *8*, 238 (1958).

Considering the importance of protein synthesis in cellular metabolism, we decided to study again the influence of chlorpromazine on this process, using a quantitative radioautographic technique. Our aim is three-fold: (1) to study the action of chlorpromazine on protein synthesis in general; (2) in each organ, to study the several cell types; (3) to disclose the influence of chlorpromazine on the cytoplasmic and nuclear uptake of the label.

Material and Methods. Seven male adult albino rats weighing 175 to 285 g were used, divided into two experiments. First, 3 animals were utilized. One was kept as control and to two others chlorpromazine was given by gastric tube for 3 days in single daily doses of 20 and 40 mg/kg body weight.

In a second experiment, 4 rats were used. One was the control, and 3 received chlorpromazine by gastric tube, for 6 days in single daily doses of 20, 40, and 80 mg/kg body weight, respectively.

To each animal, either control or chlorpromazine-treated, a single intra-peritoneal injection of 7 μc/g of body weight of ^{35}S-methionine (specific activity 45 mc/m*M*) was given. The animals were sacrificed 18 h after the injection of the labelled amino acid. Organs were fixed in Bouin fluid, paraffin embedded, sectioned at 6 micra and radioautographed with AR 10 stripping film (Kodak Ltd. London) according to the usual technique[11]. The radioautographs of the controls and the chlorpromazine-treated rats were developed at the same time, in a rack, after the appropriate exposure time (6 to 20 days). Most radioautographs were stained by basic fuchsin, through the developed film as described by BERGERON[12].

Tab. I. Effect of chlorpromazine on cellular uptake of ^{35}S-methionine. The figures represent grains counted in the photographic emulsion, per 60 μ^2 of tissue

	Control	Chlor-promazine 20 mg/kg	Chlor-promazine 40 mg/kg	Chlor-promazine 80 mg/kg
Small intestine epithelium				
villi	52.3 ± 2.95[a]	26.8 ± 3.74	20.5 ± 2.52	22.5 ± 3.48
crypt	23.5 ± 2.96	14.5 ± 2.30	11.9 ± 1.49	9.6 ± 1.50
Pancreas				
acinar cells	35.2 ± 1.81	16.2 ± 2.39	9.9 ± 1.96	12.4 ± 1.71
Langerhans islets	46.7 ± 2.49	23.6 ± 3.52	23.0 ± 3.71	22.6 ± 2.76
Submaxillary salivary gland				
acinar cells	14.3 ± 1.71	8.6 ± 1.04	—	7.1 ± 1.30
Smooth muscle	12.1 ± 1.58	9.4 ± 1.60	7.7 ± 1.42	7.3 ± 1.52

[a] Standard deviations

Tab. II. Influence of chlorpromazine on cytoplasmic and nuclear uptake ^{35}S-methionine. The figures represent silver grains over 25 μ^2 of nucleus and cytoplasm

	Control	Chlor-promazine 20 mg/kg	Chlor-promazine 40 mg/kg	Chlor-promazine 80 mg/kg
Intestinal epithelium (villi)				
nucleus	16.3 ± 1.70[a]	7.8 ± 2.25	7.8 ± 2.43	7.2 ± 1.81
cytoplasm	25.3 ± 2.01	12.4 ± 2.41	11.7 ± 2.49	12.4 ± 2.26
Pancreatic acinar cells				
nucleus	12.2 ± 1.03	4.9 ± 0.87	4.7 ± 0.67	5.5 ± 0.85
cytoplasm	14.0 ± 1.76	6.4 ± 1.96	5.7 ± 0.82	6.5 ± 1.08

[a] Standard deviations

Radioautographs exposed for 6 days were used for grain counts. With an ocular grid, an area was delimited in the film and the grains counted. For Table I a high power dry objective was used, which gave, for each square in the grid, an area of 60 μ^2. To study the nuclear uptake (Table II), an oil immersion lens was utilized, giving for each square in the grid an area of 25 μ^2. In the Table, each figure represents the average of 20 counts, and the respective standard deviation.

Results. In all chlorpromazine-treated animals, the usual behavioral changes associated with the administration of the tranquilizer were observed.

Comparing the radioautographs of the controls with the ones obtained from chlorpromazine-treated rats, it was possible to see that this drug inhibits the uptake of ^{35}S-methionine in all the organs studied. Small intestine, oesophagus, tongue, salivary gland, pancreas, liver, kidney, lung, cerebrum, cerebellum, adrenal gland, thyroid gland, and hypophysis were examined. A few typical examples are given in Table I, where we can see that some tissues are more affected by chlorpromazine than others. In small intestine, the epithelial cells lining the sides and the tip of the villi were the most affected. Next came the epithelial cells in the crypts of Lieberkühn. The influence of chlorpromazine on the ^{35}S uptake by the muscle cells was less pronounced. The pancreatic acinar cells, and the cells in the Langerhans islands were affected in a degree comparable to other glands, like hypophysis, liver, and submaxillary salivary gland.

Table II shows the influence of chlorpromazine on the nuclear and cytoplasmic uptake of ^{35}S-methionine. Counts were made over equivalent areas of cytoplasm and nucleus of the same cells. The results show that both nuclear and cytoplasmic uptake are reduced by chlorpromazine. The degree of protein synthesis inhibition produced by several doses of chlorpromazine on cytoplasm is paralleled by the nuclear inhibition.

Discussion. The incorporation of ^{35}S from methionine into animal tissues can be taken as an evidence of methionine (with a small proportion of cystine) incorporation into protein molecules[13,14].

Our results show that the administration of chlorpromazine to rats is able to reduce the uptake of ^{35}S from methionine by several tissues. This reduced uptake could be explained by several processes, e.g.: an increased protein destruction, a decreased penetration of methionine into the cells, or a decreased protein synthesis. In the light of our present knowledge, the last-mentioned process seems to be the one involved.

In our experiments it was not possible to detect a parallelism between the amount of chlorpromazine given to the animals and the inhibition of ^{35}S-methionine uptake. However, such a parallelism does occur *in vitro*, as was shown by ZÖLLER et al.[10]. Whether the pharmacological action of chlorpromazine is related to its ability partly to inhibit protein synthesis, cannot be inferred from our results.

Besides confirming the results of other authors regarding the general inhibiting effect of chlorpromazine on protein synthesis, we could observe that both nucleus and cytoplasm are affected by the drug. By examining a large number of organs, we noticed that the action of chlorpromazine is particularly pronounced in the cells in which

[11] S. R. PELC, Intern. J. Appl. Radiation and Isotopes *1*, 172 (1956).
[12] J. A. BERGERON, Stain Technol. *33*, 221 (1958).
[13] M. K. GAITONDE and D. RICHTER, Biochem. J. *59*, 690 (1955).
[14] C. P. LEBLOND, N. B. EVERETT, and B. SIMMONS, Amer. J. Anat. *101*, 225 (1957).

protein synthesis is very active, either for secretion or cell renewal. According to LEBLOND, EVERETT, and SIMMONS[14], the proteins produced in non-growing mammals meet 3 main purposes: (1) the formation of new cells in renewal systems (e.g. intestinal epithelium); (2) the elaboration of secretion (e.g. pancreas salivary gland); (3) intracellular turnover (e.g. muscle cells).

As we can see in Table I, the building of protein molecules to replace the ones broken down by the cell is less affected by chlorpromazine than the secretion and production of new cells. Thus the degree of inhibition in pancreas and intestinal epithelium cells is more pronounced than in smooth muscle cells.

The displacement of cells explains why the intestinal epithelium was found to be more radioactive in the villi than in the crypts of Lieberkühn, which is the actual site of cell renewal. As the sacrifice was 18 h after the labelling injection of ^{35}S-methionine, the cells which were in the crypts at the injection time had attained the sides of the villi by the sacrifice time[15].

When we compare the action of chlorpromazine on cytoplasmic and nuclear uptake of the labelled methionine, roughly parallel results are found (Table II). This is another instance in which a parallelism between nuclear and cytoplasmic protein synthesis is shown[16].

Résumé. Des rats ont été traités avec de la chlorpromazine et ont reçu en suite une seule injection de ^{35}S-méthionine. Des radioautographies des coupes histologiques ont montré que la chlorpromazine a un effect inhibitoire dans la synthèse de la protéine nucléaire et cytoplasmatique. Les cellules qui ont une grande vitesse de synthèse protéique sont les plus affectées.

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[15] C. P. LEBLOND, C. E. STEVENS, and R. BOGOROCH, Science *108*, 531 (1948).

[16] V. G. ALLFREY, M. M. DALY, and A. E. MIRSKY, J. gen. Physiol. *38*, 415 (1955).

[17] The authors wish to acknowledge the help of Prof. L. C. U. JUNQUEIRA for criticism and encouragement, and Miss EDNA FREYMÜLLER for her skilled technical assistance.

Oxygen Isotopic Composition of Fogs and Rains from the North Atlantic

During a research expedition in the North Western Atlantic ocean, some rain and fog samples were collected, primarily on the fishing banks of Newfoundland and Labrador, during the months of May and June 1961. All the samples have been collected on board the M/S Genepesca I.

The fog samples were collected by means of a long vertical polyethylene wire. At the lower end of this wire, the drops deposited during the movements of the ship fell in a polyethylene container. The greatest care was taken to avoid any contamination by gases from the funnel and by ocean water sprays. Since the air was saturated with water vapour and there were never strong winds during the collection of samples, it can be supposed that this method of collection did not permit any fractionation process. During the collection of the samples, the speed of the ship was rather low (3–4 miles/h).

The isotopic composition of the water samples have been measured, using the method proposed by EPSTEIN and MAYEDA[1]. The analyses have been made with a dual collector mass spectrometer of the same type described by BOATO et al.[2]. The results are shown in the Tables I and II. They are expressed in terms of difference per mil ($\delta_{O^{18}}$) of the ratio O^{18}/O^{16} between the sample and the 'mean ocean water' standard as it was defined by CRAIG[3] with the work of EPSTEIN and MAYEDA[1] as a basis.

Since the vapour pressure of H_2O^{18} is lower than that of H_2O^{16}, the O^{18} content of the atmospheric water vapour in open sea should be lower than that of the oceanic water by about $10^0/_{00}$ under equilibrium conditions. When a fraction of this vapour condenses and fog occurs, the O^{18} content of the liquid phase is higher than that of the remaining vapour. If only a small fraction of the vapour condenses, its isotopic composition will be very close to that of the oceanic water. Such a composition has been measured in samples F4 and F9. The δ values of most of the other

Tab. I. Collecting data and oxygen isotopic composition of fog samples from the North-Western Atlantic

Sample numbers	Collection date	Local time of collection	Position	Temperature (°C)	$\delta_{O^{18}}$ (SMOW)
F 1	4 june 1961	1.00 p.m.	51° 58′ N	+ 3	— 2.5
		3.00 p.m.	54° 25′ W		
F 2	10 june 1961	5.00 p.m.	53° 55′ N	0	— 3.2
		6.00 p.m.	54° 12′ W		
F 3	16 june 1961	9.00 p.m.	54° 45′ N	— 2	— 2.5
		10.00 p.m.	55° 25′ W		
F 4	19 june 1961	1.00 p.m.	54° 57′ N	+ 5	+ 0.1
		3.00 p.m.	54° 57′ W		
F 5	19–20 june	11.30 p.m.	54° 55′ N	+ 2	— 4.4
	1961	1.00 a.m.	54° 47′ W		
F 6	23 june 1961	5.00 a.m.	52° 53′ N	+ 6	— 0.8
		6.30 a.m.	54° 34′ W		
F 7	24 june 1961	3.00 p.m.	52° 16′ N	+ 6.5	— 0.9
		7.00 p.m.	54° 35′ W		
F 8	24 june 1961	8.00 p.m.	52° 10′ N	+ 2	— 3.1
		12.00 p.m.	54° 25′ W		
F 9	25 june 1961	3.00 p.m.	51° 48′ N	+ 7.5	— 0.2
		4.00 p.m.	54° 30′ W		
F 10	26 june 1961	9.00 p.m.	52° 30′ N	+ 2	— 3.3
		10.00 p.m.	53° 40′ W		
F 11	28 june 1961	9.00 p.m.	48° 53′ N	+ 5	— 5.8
		11.00 p.m.	52° 25′ W		
F 12	30 june 1961	8.00 a.m.	48° 34′ N	+ 8	— 2.6
		10.00 a.m.	50° 45′ W		
F 13	30 june 1961	8.30 p.m.	48° 10′ N	+ 7	— 1.7
		10.00 p.m.	51° 30′ W		
F 14	1 july 1961	6.00 a.m.	47° 40′ N	+ 8.5	— 2.7
		6.30 a.m.	52° 23′ W		

[1] S. EPSTEIN and T. MAYEDA, Geoch. cosmoc. Acta *4*, 213 (1953).

[2] G. BOATO, R. SANNA, M. E. VALLAURI, and M. REINHARZ, Suppl. Nuovo Cimento *16*, Ser. 10, 215 (1960).

[3] H. CRAIG, Science *133*, 1833 (1961).