the densities of Ficoll to be used by preliminary experiments on a discontinuous gradient; the densities were 1.066 for brown adipose tissue, and 1.048 for white adipose tissue. The yield of the method of KAMAT and WALLACH[8] was about 1 mg of plasma membrane proteins from 3 g of brown adipose tissue and 1 mg of plasma membrane proteins from 20 g of white adipose tissue.

The proteins were measured according to LOWRY et al.[11]. Mg^{2+}-Na^{+}-K^{+}-ATPase (EC. 3.6.1.3) activity was measured according to MODOLELL and MOORE[12]. This assay does not discriminate between the Mg^{2+}-ATPase and the Mg^{2+}-Na^{+}-K^{+}-ATPase. 5'-nucleotidase (EC. 3.1.3.5) activity was measured according to HEPPEL and HILMOE[13], inorganic phosphorus (Pi) according to TAUSSKY and SHORR[14] and NADH-diaphorase (EC. 1.6.99.3) activity, spectrophotometrically (Beckman DB-G Spectrophotometer), according to STRITTMATTER[15]. In certain experiments attempts were made to increase the yield of enzyme activity by sonicating the fractions (see Table).

Electron microscopy. Pellets obtained after recentrifugation of the sedimentation zones were fixed with 4% glutaraldehyde in 0.1 *M* phosphate buffer for 3 h. After glutaraldehyde fixation, the pellets were removed from the cellulose tubes, divided in strips when possible and postfixed in 2% osmium tetroxide. Dehydration was performed through alcohols and embedding in Epon. Ultrathin sections were stained with lead citrate and examined in a Philips EM 300 electron microscope.

Results. The marker enzymes characterizing the plasma membrane and the microsomal fractions have been measured. As shown in the Table, the Mg^{2+}-Na^{+}-K^{+}-ATPase specific activities of brown and white adipose tissues were respectively 6.0 and 4.2 times more concentrated in the plasma membrane fraction than in the microsomal fraction, while 5'-nucleotidase was found to be 5 times more concentrated in the plasma membranes than in the microsomes in both tissues. The NADH-diaphorase was about 2.5 times more active in the sonicated microsomes than in the sonicated plasma membranes.

Pellets of the plasma membrane and microsomal fractions of brown adipose tissue prepared according to the method of KAMAT and WALLACH[8] are presented in Figure 1 and 2. The plasma membrane fraction (Figure 1) is composed mostly of circular membranous profiles of varying sizes ranging from 0.08 to 0.3 μm. Several profiles appear composed of 2 or 3 superposed sheets of membranes. The microsomal pellet of the same tissue (Figure 2) is composed mostly of single-walled vesicles of small diameter (about 0.15 μm) interspersed with granular material, possibly of ribosomal origin. The lack of good morphological distinction between plasma membranes and microsomes obtained with the method of KAMAT and WALLACH[8] contrasts with the biochemical studies which indicate distinct differences between these two populations of membranes. This difference may be explained by the strong homogenization of the tissue which may disrupt the plasma membranes into small fragments. These fragments can seal up and become difficult to distinguish from the small vesicles of the microsomal fraction.

Discussion. MCKEEL and JARETT[10] showed that the Mg^{2+}-Na^{+}-K^{+}-ATPase specific activity was especially high in the plasma membranes of white adipose tissue, while this enzyme was not completely absent in the microsomes. With brown and white adipose tissues, our separation procedure provides plasma membranes highly enriched in Mg^{2+}-Na^{+}-K^{+}-ATPase activity compared with the microsomal fraction.

The 5'-nucleotidase has been used as a marker of the plasma membranes isolated from liver[4-7]. In our experiment, 5'-nucleotidase activity is distributed between plasma membrane and microsomal fractions in the same proportion as ATPase. In liver[2], ascites cells[8] and white adipose tissue[10], NADH-diaphorase is considered as a microsomal enzyme, but it has also been found in small amount in the plasma membranes. This suggests that part of the NADH-diaphorase may be an intrinsic enzyme of the plasma membranes[2,8,10]. Our data are in agreement with these observations. Our results show a great similarity between the plasma membranes of brown and white adipose tissues. Using another procedure and starting from isolated white fat cells, MCKEEL and JARETT[10] obtained a comparable distribution of the marker enzymes between the plasma membranes and the microsomes[16,17].

Résumé. Les membranes plasmatiques des tissus adipeux brun et blanc sont isolées de la fraction microsomale selon une modification de la technique décrite par KAMAT et WALLACH[8]. Les résultats de l'analyse enzymatique démontrent un degré de purification satisfaisant des membranes plasmatiques et des microsomes.

J. P. GIACOBINO and A. PERRELET

Université de Genève, Institut de Biochimie Médicale, École de Médecine, and Institut d'Histologie et d'Embryologie, École de Médecine, CH-1211 Genève 4 (Switzerland), 21 October 1970.

[11] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[12] J. B. MODOLELL and R. O. MOORE, Biochim. biophys. Acta *135*, 319 (1967).
[13] L. A. HEPPEL and R. J. HILMOE, J. biol. Chem. *188*, 665 (1951).
[14] H. H. TAUSSKY and E. SHORR, J. biol. Chem. *202*, 675 (1953).
[15] P. STRITTMATTER, J. biol. Chem. *234*, 2665 (1959).
[16] Our sincere thanks are due to Miss C. WELTER for excellent technical assistance.
[17] This work was supported by a grant from the 'Fonds national suisse de la Recherche scientifique'.

Inhibition of DNA Synthesis by Lidocaine and Procaine

We wish to report that exposure of Ehrlich ascites cells to lidocaine or procaine resulted in a preferential inhibition of DNA synthesis (Figures 1 and 2). The relationship of this observation to the anesthetic action of these drugs is unclear. It has been suggested[1], however, that local anesthetics interact with lipid components of membraneous cellular structures and it is interesting that nascent mammalian DNA has been shown to be associated with a unique cellular component, probably the nuclear membrane[2], see also[3-6]. It may therefore be that the inhibition of DNA synthesis observed by us is a result of the complexing of lidocaine and procaine with membraneous structures thereby interfering with the site of DNA synthesis.

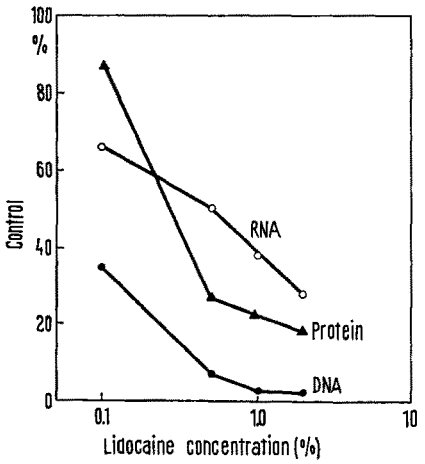

Fig. 1. Effect of lidocaine on macromolecular syntheses.

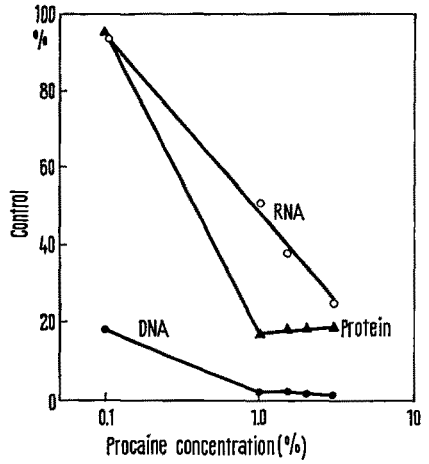

Fig. 2. Effect of procaine on macromolecular syntheses.

Ehrlich ascites cells propagated as previously described[7] were washed, resuspended in Waymouth medium (6×10^7 cells/ml) and distributed into screw-cap tubes containing premeasured amounts of the drug and radioactive precursors. [Thymidine-^{3}H for DNA ($7.7 \times 10^{-6} M$, 0.5 μc/ml); uridine-5-^{3}H for RNA ($2.0 \times 10^{-4} M$, 2.4 μc/ml); and lysine-^{3}H for proteins ($5.6 \times 10^{-4} M$, 0.3 μc/ml)]. The cultures were incubated at 37 °C for 2 h and processed for the determination of radioactivity incorporated into acid-insoluble form (Figures 1 and 2)[8,9].

Zusammenfassung. Nachweis, dass in Ehrlich-Ascites-Zellen die DNS-Synthese durch Lokalanaesthetika stärker gehemmt wird als die RNS- und die Proteinsynthese.

R. M. SCHMIDT[10], H. S. ROSENKRANZ and J. F. RYAN[11]

Departments of Microbiology and Anestesiology, College of Physicians and Surgeons, Columbia University, New York (N.Y. 10032, USA), 14 September 1970.

[1] J. C. SKOU, J. Pharm. Pharmac. *13*, 204 (1961).
[2] V. JACKSON, J. EARNHARDT and R. CHALKLEY, Biochem. Res. Commun. *33*, 253 (1968).
[3] H. G. DAVIES and J. V. SMALL, Nature, Lond. *217*, 1122 (1968).
[4] E. J. DUPRAW, Proc. natn. Acad. Sci., USA *53*, 161 (1965).
[5] D. E. COMINGS and T. J. KAKEFUDA, J. molec. Biol. *33*, 225 (1968).
[6] D. L. FRIEDMAN and G. C. MUELLER, Biochim. biophys. Acta *174*, 253 (1969).
[7] H. S. ROSENKRANZ and H. S. CARR, Cancer Res. *30*, 112 (1970).
[8] H. S. ROSENKRANZ, R. D. POLLAK and R. M. SCHMIDT, Cancer Res. *29*, 209 (1969).
[9] This investigation was supported by the Damon Runyon Memorial Fund for Cancer Research. R.M.S. was supported by Training Grant No. 1S01-FR-5395-07 from the U.S. Public Health Service and H.S.R. is a Research Career Development Awardee of the Division of General Medical Sciences, U.S. Public Health Service. The authors thank Dr. B. COVINO, Astra Pharmaceutical Co., for supplying them with lidocaine.
[10] Present address: Department of Medicine, University of California at San Diego, San Diego (Calif., USA).
[11] Present address: Department of Anaesthesia Massachusetts General Hospital, Boston (Mass., USA).

Stimulation of RNA Polymerase Activity by Histones, Polyamino Acids, and Polypeptide Hormones

DNA-dependent RNA polymerase from various sources is stimulated by polyamines and inhibited by histones and polylysine[1-5]. We wish to report that at low concentrations histones, polymers of basic amino acids and polypeptide hormones may act as polyamines and stimulate RNA polymerase of *E. coli*.

DNA-dependent RNA polymerase of *E. coli* was purchased from Biopolymers, Chagrin Falls, USA. Decalysine was a generous gift of Dr. G. L. TRITSCH, Roswell Park Memorial Institute, Buffalo, USA. RNA polymerase activity was assayed by mixing histones, polyamino acids or polypeptide hormones (0.1–20 μg/25 μl) with RNA polymerase (1.25 units/25 μl). To the mixture was added highly polymerized calf thymus DNA (20 μg/50 μl) and bovine serum albumin dissolved in 0.1 *M* *Tris*-HCl buffer, pH 7.9 (50 μg/50 μl). After incubating the mixture at 37 °C for 3 min 100 μl of a solution containing 100 μmoles each of ATP, CTP and GTP, 1 μCi of [^{3}H]UTP, 1.25 μmoles of $MgCl_2$, 0.5 μmoles of $MnCl_2$, 1.0 μmoles of β-mercaptoethanol, 3.0 μmoles of *Tris*-HCl buffer (pH 7.9) were added and incubated at 37 °C for 10 min. The reaction was terminated by the addition of 2.5 ml of cold 5% TCA. The precipitate was washed 3 times, heated at 100 °C to dryness, and dissolved in 0.5 ml of Soluene 100 (Packard Instrument Co.) overnight. Radioactivity was measured as previously described[6].

Figure 1 shows the effect of calf thymus histones on the RNA polymerase activity. At high concentrations, histones inhibited the polymerase activity as was reported by FOX and WEISS[1]. However, when less than 4.0 μg was

[1] C. F. FOX and S. B. WEISS, J. biol. Chem. *239*, 175 (1964).
[2] J. BONNER, M. E. DAHMUS, D. FAMBROUGH, R-C. C. HUANG, K. MARUSHIGE and D. Y. H. TUAN, Science *159*, 47 (1968).
[3] T. C. SPELSBERG and L. S. HNILICA, Biochim. biophys. Acta *195*, 55 (1969).
[4] T. C. SPELSBERG, S. TANKERSLEY and L. S. HNILICA, Proc. natn. Acad. Sci., USA *62*, 1218 (1969).
[5] E. FUCHS, W. ZILLIG, P. H. HOFSCHNEIDER and A. PREUSS, J. molec. Biol. *10*, 546 (1964).
[6] K. SUNAGA and S. S. KOIDE, J. pharm. Sci. *57*, 2116 (1968).