in the normal preparation. However, an examination of cell c (normal) and d (perfused) would indicate that the reverse was true.

The membrane potential of perfused and normal cells was held constant during excitation while the membrane current was measured with the circuit shown in Fig. 1. In perfused cells, the inflow pipette contained a platinized silver chloride electrode which was used to pass current down the vacuole. Initially under these conditions, I was unable to confirm any marked difference in the time course of the membrane current between the two. There does appear to be a delay in the outward current from perfused cells, but the data are not sufficient to warrant firm conclusions (Fig. 2B). The vacuolar composition of *Nitella* can be continuously controlled without detriment to either the tonoplast or the plasmalemma.

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DNA Polymerase Activities Associated with Smooth Membranes and Ribosomes from Rat Liver and Hepatoma Cytoplasm

Abstract. *Deoxyribonucleic acid polymerase activity that prefers denatured DNA primer concentrates with the smooth membranes during sucrose gradient centrifugation of rat liver and hepatoma cytoplasmic extracts. The activity in this fraction is eightfold higher in hepatoma tissue. Deoxyribonucleic acid polymerase activity that prefers native DNA primer concentrates with the free ribosome fraction from both tissues.*

Most investigations of DNA polymerase in normal rat liver and hepatomas, with the exception of a few specific studies of nuclear (*1*) or mitochondrial (*2*) DNA polymerase, have utilized the postmicrosomal supernatant solution as the starting material for enzyme isolation. In general, these studies have shown that DNA polymerase activity present in the postmicrosomal supernatant solution of normal adult and regenerating rat liver has a preference for native DNA as primer and is of relatively low molecular weight (*3*). It has recently been reported that the postmicrosomal supernatant solutions from fetal rat liver and hepatomas also contain a high-molecular-weight DNA polymerase with a preference for denatured DNA primer (*4*). The results presented here show that the latter DNA polymerase activity can be isolated from the postmicrosomal supernatant solution of normal adult rat liver, as well as hepatoma tissue. This activity is associated with the smooth membrane fraction in both tissues, but the activity is higher in hepatoma. By contrast, the DNA polymerase activity that prefers native DNA primer is concentrated in the free ribosome fraction obtained from either liver or hepatoma.

Morris hepatoma 7777 (generation No. 55) was transplanted intramuscularly in male, Buffalo strain rats at Howard University, Washington, D.C., and the animals were shipped by air express. Normal (Holtzman) and tumor-bearing rats were permitted to feed ad libitum, but were fasted overnight before sacrifice. A 20 percent homogenate of the perfused liver or hepatoma was prepared in 0.25*M* sucrose-TKMM (0.05*M* tris-HCl, *p*H 7.5 at 20°C, 0.025*M* KCl, 0.005*M* $MgCl_2$, 0.001*M* 2-mercaptoethanol) as described by Blobel and Potter (*5*). Nuclei (P-1) and mitochondria (P-2) were removed by successive centrifugation at 600*g* for 10 minutes and 20,000*g* for 15 minutes. The postmitochondrial supernatant solution (S-2) was centrifuged at 105,000*g* for 1 hour to remove microsomes (P-3). The postmicrosomal supernatant solution (S-3) was centrifuged at 105,000*g* for 15 hours to assure complete sedimentation of the free ribosomes and smooth membranes and thereby to separate these particulates as completely as possible from soluble materials. The resultant solution (S-4) was removed by aspiration, and the pellet (P-4) was resuspended in TKMM buffer by ten strokes with a loose-fitting Dounce homogenizer. The free ribosome and smooth membrane fractions were separated in a discontinuous sucrose gradient (0.8 to 2.0*M* sucrose in TKMM buffer) prepared according to Murray *et al.* (*6*). Centrifugation was performed in the No. 30 rotor at 30,000 rev/min for 24 hours. The sucrose layers were carefully removed with a pipette and diluted with three volumes of TKMM buffer. The pellet (free ribosomes) was resuspended in TKMM buffer by ten strokes with a loose-fitting Dounce homogenizer. The resuspended pellet and the diluted sucrose layers were recentrifuged at 105,000*g* for 15 hours. The supernatant solutions (soluble material) were decanted and the free ribosome and membrane pellets were drained and resuspended in TKMM buffer. All procedures were carried out at 0° to 4°C.

Deoxyribonucleic acid polymerase activity in the postmicrosomal supernatant solution (S-3) of normal rat liver is low and has no preference for native or heat-denatured DNA as primer (Table 1). In contrast, S-3 from hepatoma tissue has a higher polymerase activity, and heat-denatured DNA is preferred as primer, as previously reported (*4*).

Centrifugation of the S-3 from either normal liver or hepatoma at 105,000*g* for 15 hours concentrates the denatured primer–preferring activity in the pellet (P-4). The specific activity with heat-denatured DNA primer remained considerably higher in the hepatoma preparation, whereas the specific activity with native DNA primer was equal in P-4 fractions isolated from liver and hepatoma tissue. The supernatant solution (S-4) from hepatoma exhibited higher polymerase activity with native DNA primer than did S-4 from liver.

Sucrose gradient centrifugation of resuspended P-4 from liver or hepatoma separated the DNA polymerase activity into three fractions: that associated with the free ribosomes which sedimented through the 2.0*M* sucrose layer, that with the membrane fraction that banded at the interface of the 0.8 to 1.3*M* sucrose layers and subsequently pelleted after dilution and recentrifugation for 15 hours, and that in the soluble fraction from the gradient that did not pellet after dilution and recentrifugation for 15 hours. Nearly all of the denatured primer–preferring polymerase activity in the P-4 fractions from liver or hepatoma tissue was concentrated with the membrane fraction after sucrose gradient centrifugation. However, the

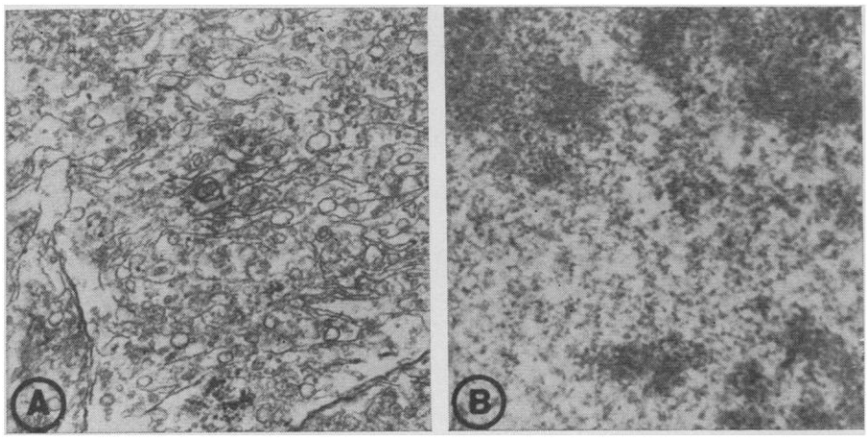

Fig. 1. Electron micrographs of the subcellular components found in the sucrose gradient fractions in which DNA polymerase activities with preference for native or heat-denatured DNA primers were concentrated. (A) Section of pellet obtained after dilution and centrifugation at 27,000 rev/min (No. 30 rotor) for 15 hours of the band located between the 1.3*M* and 0.8*M* sucrose layers of the gradient. This fraction is composed mainly of smooth membranes. (B) Section of the pellet obtained by material passing through the 2*M* sucrose layer of the gradient and consisting almost entirely of free ribosomes. Pellets were fixed in buffered 2 percent OsO_4, embedded in epon, and sections stained with lead citrate–uranyl acetate. (× 27,400).

activity with denatured DNA primer was about eightfold greater in the membrane fraction from hepatoma than from normal liver. Polymerase activity with native DNA as primer was similar in the membrane fractions from both liver and hepatoma. Deoxyribonucleic acid polymerase activity was occasionally observed at the interphase of the 1.3 to 2.0*M* sucrose layers of the gradient in fractionations of P-4 pellets from rat liver. This activity showed no preference for native or denatured DNA primer and is presumably due to DNA polymerase associated with rough endoplasmic reticulum.

Free ribosomes, obtained by sucrose gradient centrifugation of P-4, also contained polymerase activity that preferred native DNA as primer, although the activity was three times higher in free ribosomes from hepatoma. The yield of free ribosomes from the hepatoma was four to five times greater than from liver, although the yield of membrane was only slightly increased for the hepatoma. Thus, the increased polymerase activity with these fractions from hepatoma tissue represents an increased total activity. Liver from tumor-bearing rats gave results similar to those obtained with normal rat liver, in agreement with previous findings for host-liver DNA polymerase activity reported by Ove *et al.* (*4*).

The postmicrosomal supernatant solution of rat liver was found to contain 45 to 50 percent of the total DNA polymerase activity present in the crude homogenate. Most (90 to 95 percent) of the denatured DNA primer–preferring activity of the post microsomal supernatant solution of rat liver and hepatoma sedimented after 15 hours of centrifugation at 105,000*g*. However, differences in the distribution of nuclease activity, as well as DNA polymerase activity, make calculations of total DNA polymerase activities tenuous. Deoxyribonuclease present in the membrane and ribosome fractions digests both native and denatured DNA, and preincubation of the fractions with primer DNA did not alter the subsequent preference for native or denatured DNA primer (*7*). Although it seems unlikely that the primer preferences observed with these fractions are solely determined by the action of nucleases, further experiments are required to establish this point.

Maximal activity of DNA polymerase in the membrane and ribosome fractions requires the addition of Mg^{2+}, DNA, and a sulfhydryl reducing agent. The DNA polymerase associated with the membrane fraction requires all four deoxyribonucleoside triphosphates. Maximal activity was also observed in the ribosome fraction with native DNA primer only if all four deoxyribonucleoside triphosphates were present. However, in the absence of deoxyguanosine triphosphate (dGTP) and deoxycytidine triphosphate (dCTP), 50 percent of the incorporation obtained with the complete system was observed. This suggests the presence of terminal nucleotidyltransferase in addition to DNA polymerase activity in the ribosome fraction. However, this terminal nucleotidyltransferase also has a preference for native DNA primer, and preliminary experiments with deoxyribohomopolymers indicate that addition of single deoxyribonucleotides by this enzyme requires base pairing with the primer.

The sucrose gradient fractions were examined for homogeneity by electron microscopy (*8*). The band formed between the 0.8 and 1.3*M* sucrose layers was composed entirely of smooth membrane elements (Fig. 1A) with minimal or no contamination by intact nuclei, mitochondria, or ribosomal elements. Chemical analyses of this fraction showed an RNA/protein ratio of 0.06, a phospholipid content of 280 μg/mg of protein (*8*), and no detectable DNA. The low RNA/protein ratio and high

Table 1. Deoxyribonucleic acid polymerase activity in subfractions derived from the postmicrosomal supernatant solution from normal rat liver and Morris hepatoma 7777. The procedures for the assay were as previously described by Ove *et al.* (*3*). Incubation was in a volume of 0.5 ml at 37°C for 60 minutes. The incubation contained 20 μmole of glycine-NaOH buffer (*p*H 8.0), 8 μmole of $MgCl_2$ 0.5 μmole of 2-mercaptoethanol, 0.05 μmole each of dATP (deoxyadenosine triphosphate), dGTP, and dCTP, [^{3}H]TTP (5 μc/μmole), 0.1 mg of calf thymus DNA, and 0.1 to 0.2 mg of protein from each fraction. The results are the average of four determinations with a variation of less than 10 percent. N indicates native DNA primer and D indicates heat-denatured DNA primer.

Substance	Nanomoles of [^{3}H]TTP incorporated per milligram per hour			
	Normal liver		Hepatoma 7777	
	N	D	N	D
Postmicrosomal supernatant (S-3)	0.83	0.58	3.16	7.97
Sucrose gradient subfractionation of P-4				
Pellet (P-4)	1.59	1.90	1.65	6.72
Supernatant (S-4)	0.48	0	1.46	0.84
Sucrose gradient subfractionation of P-4				
Soluble material	0.26	0	0.41	0.68
Membranes	1.22	2.84	1.76	21.76
Free ribosomes	1.23	0.26	3.3	1.3

phospholipid content lend support to the electron microscopic evidence that this fraction is composed of smooth membranes. Electron microscopy of the pellet formed below the 2*M* sucrose layer showed that this was composed of free ribosomes (Fig. 1B). There was no apparent contamination by nuclei, mitochondria, or heavy membrane elements.

Deoxyribonucleic acid polymerase activity can be dissociated from the free ribosomes, but not the smooth membrane fraction, in the presence of 0.2*M* KCl or NH_4Cl. The ribosome and membrane-associated enzymes have been purified about 1300- and 400-fold, respectively, over the activity measured in the postmitochondrial supernatant solution (*7*). Gel-filtration on Sephadex G-200 indicates that the membrane-associated DNA polymerase is of relatively high molecular weight, whereas the ribosome-associated polymerase is of lower molecular weight. The latter behaves like a basic protein during chromatography on diethylaminoethyl cellulose and phosphocellulose, whereas the membrane-associated activity appears to be more acidic. Thus, the two fractions display differences in both physical and enzymatic properties.

There is still a question as to whether the distribution of these enzymes is an artifact produced during the fractionation. The ability to dissociate the enzyme activity from the free ribosomes by 0.2*M* KCl or NH_4Cl might be suggestive of nonspecific association (*9*), although this salt concentration has recently been used to dissociate protein factors from the bacterial ribosomes of which they are considered to be an integral part (*10*). Also, fractionation of normal rat liver with different homogenization and extraction conditions produced similar qualitative results (*7*). The requirement for exogenous DNA and the denatured DNA primer preference of the membrane-associated polymerase activity suggest that this enzyme differs from the mitochondrial membrane-associated polymerase reported by Schultz *et al.* (*2*). However, comparative studies of the physical and enzymatic properties of the enzymes reported here and those of rat liver nuclear and mitochondrial DNA polymerase are needed.

The results presented here do demonstrate that the DNA polymerase activity with a preference for denatured DNA primer, previously reported to be markedly increased in the postmicrosomal supernatant solution from hepatomas (*4*), is associated with the smooth membrane fraction. A similar but lower DNA polymerase activity is also associated with this subcellular fraction from normal rat liver. Deoxyribonucleic acid polymerase activity with a preference for native DNA primer is associated with the free ribosome fraction from both liver and hepatoma tissue.

The significance of the association of different DNA polymerase activities with these cytoplasmic fractions and the increased activity in hepatomas has not yet been defined. The alterations in the endoplasmic reticulum (*11*) and distribution of ribosomes (*12*) that occur in hepatomas could certainly influence this apparent association when the same methodological approach is applied to liver and hepatoma tissues. Furthermore, it is of interest that an association of DNA with microsomes and polysomes has recently been reported (*13*), although the function and origin of this DNA remains obscure at the present time (*14*).

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Drug Effect Prediction by Computer

Abstract. *The mass of information available about effects of chemical substances (drugs) on behavioral, biochemical, and physiological systems of living organisms is so extensive as to defy traditional methods of analysis. A procedure that provides automated, computerized searches for patterns among these effects has been developed and has been applied to a data base constructed of medical and chemical information from* The Merck Index. *One promising result is the development of new hypotheses about mechanisms of drug action.*

A considerable amount of information about the behavioral effects of chemical substances is, in a practical sense, buried in medical literature and is therefore neglected. Several factors contrive to create this situation. (i) Knowledge of the effects of drugs, based upon accumulated medical experience, is so vast as to be unmanageable without systematic means for accessing (or retrieving) it. (ii) Traditional methods of search, even though systematic, require expenditures of time and money that are frequently unreasonable. (iii) Behavioral effects are often codified in terminology that lacks precision in terms of psychologically meaningful dimensions. (iv) New knowledge about drugs tends to be related to specific problems with little opportunity for generalizing. (v) A number of scientific disciplines are involved in drug analysis, and interpretations across multidisciplinary lines are difficult.

Advances in computer technology have increased the feasibility of developing automated search routines (software packages) that permit gathering information from large bodies of data without an intermediate encoding process. The costs of accessing such natural-language data bases are modest enough to justify conducting inquiries on a broad scale, even in cases where payoff potential may be low. In fact, the new computer systems have again