The DPNH was prepared from DPN 'Sigma' by enzymatic reduction with alcohol and alcohol dehydrogenase, crystallized according to RACKER[6].

Table I

PHI activity of perilymph, liquor and serum expressed in mg F6P formed per hour

	Perilymph	Liquor	Serum
mg F6P/ml/h .	2·51 ± 0·1	0·53 ± 0·07	1·08 ± 0·04
mg F6P/mg protein/h . .	0·82 ± 0·016	0·36 ± 0·05	0·016 ± 0·001

In a cuvette of the Beckman spectrophotometer were mixed: 0·48 μM DPNH in 0·1 ml; 3 μM piruvate in 0·1 ml; 0·3 ml $NaHCO_3$ 0·02 Mol; 2·5 ml phosphate buffer 0·1 Mol, pH 7·8. The reaction was started by adding 0·25 ml of the fluid under examination, and the decrease in optical density followed, at room temperature, for 10 min, taking readings every minute.

Proteins were determined in the fluids before each enzymatic test by GOA's micromethod[7].

Table II

LD activity of perilymph, liquor and serum expressed in Mol × 10^7 lactate formed per minute

	Perilymph	Liquor	Serum
Mol × 10^7 lactate/ml/min .	1·75 ± 0·07	0·22 ± 0·03	2·63 ± 0·2
Mol × 10^{-7} lactate/mg protein/min . .	0·58 ± 0·03	0·15 ± 0·025	0·04 ± 0·003

Results. In Table I and II the values for PHI and LD activities in perilymph, liquor and blood serum are reported. From these data it is evident that PHI and LD activities are present in the perilymph to a considerable degree. With reference to the protein content, much higher activities are found for perilymph than for liquor and serum. The activities for milligrams of protein are in perilymph, liquor and serum in the ratio 100:44:2 for PHI, and 100:26:7 for LD.

These results contribute to differentiate the perilymph from both blood serum and cerebro-spinal fluid.

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Riassunto

È stata messa in evidenza nella perilinfa di cavallo la presenza di notevole attività fosfoesosoisomerasica e latticodeidrogenasica. In rapporto al contenuto proteico tali attività enzimatiche risultano maggiori nella perilinfa che nel siero di sangue e nel liquor.

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Physical Properties of Deoxyribonucleic Acid Isolated from Ehrlich Ascites Tumor Cells by Various Methods

The requirement for tumor cell deoxyribonucleic acid (DNA) to be used in certain biological studies in progress in this laboratory led to an examination of various methods for the isolation of this cell component.

The Ehrlich ascites carcinoma was employed throughout the investigations. DNA has been isolated from cells of this tumor by four different methods, two of which involved the use of surface active agents. In addition, DNA has been separated from deoxyribonucleoprotein isolated from this tumor by means of chloroform-octyl alcohol and by extraction with hot concentrated salt solution.

All samples were subjected to analysis for nitrogen and phosphorus, and a number of the physical properties of all samples were measured. A description of these physical properties comprises the main subject of this report.

Experimental. The Ehrlich ascites carcinoma was grown in Swiss albino mice. Seven to nine days after the intraperitoneal implantation of 10–12 × 10^6 tumor cells, the mice were sacrificed and ascitic fluids aspirated from the peritoneal cavities. The cells were freed of erythrocytes by washing in physiological saline in the manner described by MCKEE, LONBERG-HOLM, and JEHL[1]. The washed cells were transferred to a mortar set in an alcohol-dry ice bath, and when thoroughly frozen were ground to a fine, homogeneous powder.

The powder was placed in a Waring blendor containing isotonic sodium chloride, pH 7·0 and 0·001 M with respect to sodium citrate, and was blended at low speed for 10–15 min. Nuclei were sedimented from the suspension by centrifugation at 2000 rpm for 20 min. The supernatant was discarded and the nuclei washed three additional times in saline-citrate solution.

DNA was isolated directly from such nuclei by the sodium xylene sulfonate method of SIMMONS, CHAVOS, and ORBACH[2] (SCO) and the sodium dodecyl sulfate method of KAY, SIMMONS, and DOUNCE[3] (KSD).

Two more samples were obtained by first isolating deoxyribonucleoprotein from the nuclei by a technique derived from the methods of STERN[4] and GAJDUSEK[5]. In brief, this consisted of extracting the nucleoprotein by shaking the nuclei for 16–24 h in the cold with distilled water, precipitating the protein from the filtered extract at 0·14 M sodium chloride concentration, and further purifying it by three cycles of dissolution in distilled water and precipitation at isotonic sodium chloride concentration.

One sample of DNA was isolated from the nucleoprotein by the MIRSKY and POLLISTER[6] modification of Sevag's chloroform-octyl alcohol technique[7]. A second was prepared by extracting the protein with 10% sodium

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chloride solution, pH 7·4, as described by TYNER *et al.*[8]. DNA was precipitated from the chilled extract by the addition of 2 volumes of 95% ethanol, after which it was washed with ethanol and acetone and dried. Finally the nucleate was put in solution in distilled water, dialyzed for several days against frequent changes of distilled water and separated by lyophilization.

Table I

Sample	Drying method	% N	% P	N/P
SCO	*V**	13·81	8·45	1·63
	*O***	13·63	8·38	1·63
	*A****	14·35	8·77	1·64
KSD	*V*	14·05	8·40	1·67
	O	14·04	8·38	1·68
	A	14·34	8·50	1·69
C–O	*V*	13·27	8·10	1·64
	O	12·86	7·95	1·62
	A	13·11	8·10	1·62
NaCl	*V*	14·51	8·28	1·75
	O	14·71	8·32	1·77
	A	14·90	8·60	1·73

* Vacuum desiccator (96 h, 60–80 mm/25°C) ** Oven (48 h, 65°C) *** Abderhalden (96 h, 3 mm/80°C)

The samples were dried over calcium chloride in a vacuum desiccator (60–80 mm) for 96 h, in an oven at 65°C for 48 h, and in the Abderhalden apparatus for 96 h using P_2O_5 as the desiccant and boiling benzene (80°C) as a source of heat. The apparatus was operated at 3 mm. Nitrogen was determined by Nesslerization following sulfuric acid-hydrogen peroxide digestion, and phosphorus by the method of FISKE and SUBBAROW[9].

Specific absorption was measured in a Beckman spectrophotometer, model DU, and electrophoretic mobility was studied in a Spinco model H electrophoresis-diffusion apparatus. Sedimentation data were obtained from the Spinco model E analytical ultracentrifuge, and viscosity data by means of the Ostwald viscometer. Samples used for physical measurements were dissolved in a buffer containing 0·1 *M* KCl and 0·02 *M* NaOH which had been adjusted to pH 6·4 with cacodylic acid. Solutions were dialyzed against the same buffer before use.

Results and Discussion. The data are presented in Tables I and II. Each value represents the mean of several determinations. The method of drying had no effect on the N/P ratios of the products. This would be expected, as would the somewhat higher N and P values obtained for these samples dried in the Abderhalden apparatus.

Measuring the absorption of thymus DNA in 0·2 *M* NaCl, REICHMAN *et al.*[10] found E 1%/1 cm to be 213. Our determinations were carried out at $\Gamma/2 = 0{\cdot}12$ which should yield somewhat higher values. This appears to be the case for sample SCO.

KATZ and SCHACHMAN[11] measured the sedimentation constant of thymus DNA prepared by the method of

Table II

Sample	$E^{1\%}_{1\text{ cm}}$ (260 mμ)	μ (× 10^5)	$S^{H_2O}_{20°}$				$[\eta]c$ o
			0·6%	0·4%	0·2%	0·1%	
SCO	218	19·4	4·14	4·82	5·88	7·74	35·4
KSD	208	16·6	4·71	5·69	7·30	11·0	20·3
C–O	203	11·7	4·60	5·43	6·18	7·06	3·13
NaCl	224	15·1	—	6·90	—	—	2·08

SCHWANDER and SIGNER[12], a preparation which has recently been described by KAWADE and WATANABE[13] as being an excellent one. Their value, at 0·03% concentration, was S = 11. At 0·03% concentration, the sedimentation constant of our sample SCO would be close to 11, and that of sample KSD would be appreciably higher.

Viscosity measurements were made in an Ostwald viscometer at 20°C with an average shear rate of 350 s^{-1} as calculated from the formula of KROEPLIN[14]. At this shear rate SCHWANDER and SIGNER's preparation had a reduced viscosity of 25 at 15 mg% concentration. The values given in Table II were obtained by extrapolating to zero concentration. At a concentration of 15 mg%, the reduced viscosities of samples SCO and KSD would be 44 and 22 respectively. Inasmuch as our determinations were carried out in a medium of lower ionic strength (0·12 as compared to 1·7 employed by SCHWANDER and SIGNER[12]), it might be more valid to compare our extrapolated values with the reduced viscosity measured at a concentration of 15 mg% by SCHWANDER and SIGNER[12].

In general, the material prepared by the sodium xylene sulfonate method of SIMMONS, CHAVOS, and ORBACH[2] seemed to give the most highly polymerized DNA, and to be comparable to the best preparations reported in the literature.

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Zusammenfassung

Die Desoxyribonukleinsäure des Ehrlich-Karzinoms wurde nach vier verschiedenen Methoden isoliert und Stickstoff- und Phosphorgehalt sowie die physikalischen Eigenschaften für jedes der vier Präparate bestimmt. Die mit Hilfe von Natrium-Xylolsulfonat isolierte Desoxyribonukleinsäure scheint am stärksten polymerisiert zu sein.

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