that red cells subjected to a marked osmotic gradient agglutinate and thus produce obstruction to blood flow by a process of microthromboembolism. The present findings are consistent with the hypothesis that this phenomenon is responsible for renal destruction arising from concentrated solutions.

Summary. Laparotomy was performed on 36 mongrel dogs. The aorticorenal segment was isolated and perfused with either blood or plasma. One ml/kg of 12.5% sodium chloride or 25% urea in 0.9% saline was injected during the perfusion. Concentrated sodium chloride produced extensive damage in the blood perfused organ but not when plasma was used. Kidneys injected with 25% urea in 0.9% saline regardless of perfusate did not differ from control organs or from each other. Animals whose only functioning kidneys were injected with sodium chloride developed uremia if blood was used for perfusion. It is concluded that the renal toxicity of concentrated solutions is dependent to a considerable degree upon the presence of the red cell and is not therefore a primary effect upon the renal parenchyma. The importance of the red cell under these circumstances may be related to the phenomenon of intravascular agglutination which has been described previously in other areas of the body following administration of markedly hyperosmotic solutions.

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Received March 3, 1961. P.S.E.B.M., 1961, v107.

Mitochondrial Swelling Produced by Compounds Exhibiting Estrogenic Activity.* (26568)

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The observation that the synthetic estrogen, diethylstilbesterol, when given together with carcinostatic levels of the niacin antagonist, 6-aminonicotinamide, produces regression in the 755 tumor grown in C_{57} black mice(1) coupled with the demonstration that diethylstilbesterol greatly increases the ability of 6-aminonicotinamide to antagonize various pyridine nucleotide-dependent multienzyme systems(1,2) led us to determine the effect of diethylstilbesterol and other synthetic or natural phenolic compounds possessing estrogenic activity on mitochondrial volume or structure. The report of these findings is the purpose of this communication.

Materials and methods. Mitochondria were isolated from the livers of non-fasted male Holzman rats weighing 250-350 g. Preparation of the mitochondria and the technic employed in measuring mitochondria swelling were essentially that of Tapley(3) except that a Beckman DU spectrophotometer was employed. Inhibitory compounds of reagent grade were dissolved in 95% ethanol immediately before use and were shielded from sunlight. Ethylenediaminetetraacetic acid (EDTA), Mg^{++} and adenosine triphosphate(ATP) were dissolved in the concentrations indicated and adjusted to pH 7.4 before use. Three times glass-distilled water was used throughout.

Results. The stability of rat liver mitochondrial preparations in various concentrations of sucrose with and without diethylstilbesterol is presented in Table I. The results

* Supported by a grant and a Senior Research Fellowship from U. S. Public Health Service.

TABLE I. Effect of Diethylstilbesterol on Mitochondrial Swelling in Various Concentrations of Sucrose.*

Medium addition	Swelling, △ O.D. × 10^3 at 520 mμ △ 10′	△ 20′	△ 30′
.5 M sucrose	−2	1	0
Idem + diethylstilbesterol	170	220	265
.3 M sucrose	15	35	85
Idem + diethylstilbesterol	160	225	275
.05 M sucrose	270	290	300
Idem + diethylstilbesterol	250	270	275

* Diethylstilbesterol was dissolved in 95% EtOH, so that 0.1 ml when diluted to a total volume of 3.0 ml gave a final molarity of 8.0×10^{-5}M. An equal volume of 95% EtOH was added to the control reactions.

pertaining to mitochondrial stability in sucrose solutions of various strengths are in agreement with those found by others(3,4,5). Mitochondrial preparations were unaffected by 0.5M sucrose. However, in a medium consisting of 0.3M sucrose slow but steady swelling of the mitochondria occurred and in 0.05M sucrose a very rapid swelling was observed. Diethylstilbesterol at a final concentration of 8.0×10^{-5}M accelerates swelling in presence of 0.3M sucrose, but has no effect on the rapid swelling which results when the mitochondria are suspended in 0.5M sucrose. The effect on the swelling of rat liver mitochondria by various compounds with estrogenic activity is presented in Table II.

TABLE II. Effect of Various Estrogenic Compounds on Volume of Rat Liver Mitochondria.*

Medium addition	Swelling, △ O.D. × 10^3 at 520 mμ △ 10′	△ 20′	△ 30′
None	2	7	2
EtOH	1	3	6
Estradiol (8.0×10^{-5}M)	2	3	4
Phloretin (8.0×10^{-5}M)	1	2	5
Diethylstilbesterol			
(4.0×10^{-5}M)	30	62	82
(8.0×10^{-5}M)	160	220	270
Hexosterol (4.0×10^{-5}M)	32	62	84
" (8.0×10^{-5}M)	158	216	268
Dienesterol (4.0×10^{-5}M)	45	80	110
" (8.0×10^{-5}M)	165	230	290

* Mitochondria were suspended in 0.5 M sucrose. All test compounds were dissolved in 95% EtOH, so that 0.1 ml, in a total volume of 3 ml, gave the final molarities indicated.

Estradiol and phloretin at concentrations high as 8.0×10^{-5}M had no apparent e on mitochondrial volume. It was impossi to determine accurately the effect of high concentrations of these compounds owing to their insolubility in an aqueous medium. Diethylstilbesterol, hexosterol and dienesterol at concentrations of 4.0×10^{-5}M produced significant increases in the volume of rat liver mitochondria. Increasing the levels of these 3 synthetic estrogens to 8.0×10^{-5}M markedly increased rate of swelling. No significant difference in the relative ability of either hexosterol, diethylstilbesterol or dienesterol to cause mitochondria swelling was observed. Neither magnesium, ATP (Table III) nor

TABLE III. Effect of ATP, Magnesium and Versene on Rat Liver Mitochondrial Volumes Changes Produced by Diethylstilbesterol.*

Medium addition	Swelling, △ O.D. × 10^3 at 520 mμ △ 10′	△ 20′	△ 30′
EtOH control	−2	1	2
Diethylstilbesterol	169	217	263
Mg^{++}	0	1	3
" + diethylstilbesterol	168	215	260
ATP	1	1	2
" + diethylstilbesterol	169	216	260
Versene	0	1	6
" + diethylstilbesterol	169	217	262

* Mg^{++} as $MgCl_2$, disodium salt of ATP, and EDTA (1.0×10^{-3}M) final concentration were dissolved in 0.5 M sucrose and neutralized to pH 7.4 before addition to the reaction vessels. Mitochondria were suspended in 0.5 M sucrose. Diethylstilbesterol was added in 95% EtOH to a final molarity of 8.0×10^{-5}M. An equivalent amount of EtOH (0.1 ml) was added to all other reaction vessels.

EDTA had an effect on the ability of diethylstilbesterol to produce mitochondria swelling.

Discussion. These studies employing liver confirm the observations of Packer and Bacila(6) that diethylstilbesterol causes swelling of heart sarcosomes. The effect of diethylstilbesterol, dienesterol and hexosterol on the volume of hepatic mitochondria differs a bit from the mitochondrial volume changes produced by desoxycortisone, progesterone and other natural steroids on liver and tumor mitochondria(7). In the presence of progesterone and other natural steroids the mitochondria volume changes can be reversed by magnesium or ATP(7). In our studies mag-

ium or ATP were without effect in reversing the effects of diethylstilbesterol, dienesterol or hexosterol on mitochondrial swelling. These relatively large quantities of inhibitors needed to produce significant changes in mitochondrial volume together with a lack of correlation of the relative abilities of these estrogenic substances to produce swelling and potentiate 6-aminonicotinamide toxicity on the 755 tumor[1] apparently discount possible *in vivo* changes in mitochondrial volume, produced by these compounds, as being pertinent to the biological results. Thus, subsequent increases in mitochondrial permeability to 6-aminonicotinamide or pyridine nucleotide analogues containing 6-aminonicotinamide may be ruled out as a major factor in enhancing 6-aminonicotinamide antagonism of intramitochondrial enzymes dependent on pyridine nucleotide.

[1] Diethylstilbesterol and hexosterol are far more effective than estradiol, dienesterol and phloretin in potentiating the anti-tumor activity of the niacin antagonist 6-aminonicotinamide in the 755 tumor grown in 755 black mice (unpublished data, Dietrich, L. S. and Martin, D. S.).

Summary. The ability of the compounds diethylstilbesterol, hexosterol, dienesterol, phloretin and estradiol to induce swelling of liver mitochondria was determined. Diethylstilbesterol, hexosterol and dienesterol produced marked mitochondrial swelling at final concentration of 4-8 $\times$ 10^{-5}M. The compounds estradiol and phloretin were inactive at the highest levels tested (8 $\times$ 10^{-5}M). Under the experimental conditions ATP, Mg^{++} or versene did not prevent the mitochondrial swelling induced by diethylstilbesterol.

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Received March, 15, 1961. P.S.E.B.M., 1961, v107.

Proteins in Normal Cerebrospinal Fluid Not Found in Serum.* (26569)

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Recent reports dealing with proteins in the cerebrospinal fluid (CSF) have neither proved nor disproved the existence of proteins specific for CSF and not found in serum. Paper electrophoresis(1,2,3) does not demonstrate antigenic differences between the 2 fluids. The commercially available antiserum against human serum proteins has been used in several immuno-electrophoretic investigations of CSF (see survey by Burtin(4) and by Clausen (5)). These investigations, however, did not reveal the existence of special CSF proteins not found in serum. Immuno-electrophoresis using specific sera against CSF is a reliable method of demonstrating differences between proteins of CSF and proteins of serum. This communication adds some data to the very few results obtained in this field(6).

* Supported by the Danish League Against Multiple Sclerosis, Copenhagen, Denmark.

Materials and methods. Normal cerebrospinal fluids were obtained from patients who had undergone pneumo-encephalography at The Municipal Hospital, Copenhagen, Denmark. These patients had no other neurological complaints than cephalalgia, without objective findings.

Immuno-electrophoresis was performed as described by Scheidegger(7). The antisera made in this laboratory were manufactured as described previously(8). The following antisera were used: 1) Rabbit anti CSF anti-