No loss of inhibition resulted. In some DLE sera not giving a clot with DNA following addition of the cobalt salt, there was precipitate after 12 hours of standing at 4°C. This precipitate was no greater in those sera to which DNA *and* the cobalt salt had been added, than in those to which DNA alone had been. These precipitates, soluble in 1M NaCl, reacted strongly in capillary precipitin tubes with anti-human gamma globulin. Presumably these resembled the DNA-DLE gamma globulin precipitates described by Deicher *et al.*(4).

Discussion. There are 2 possible explanations for DNA clot inhibition following the addition of hexamminecobaltic chloride. Either the DNA could be depolymerised (as in the case of prior incubation with DNase) or the anionic groups of the polynucleotide could be linked by salt-like bonds to a factor in positive sera making these groups unavailable to the polyvalent cobalt cation. The latter possibility is supported by inhibition of DNA clot formation following addition of basic proteins like histone and protamine to normal serum. Failure of EDTA alone to block inhibition, and studies suggesting removal of the reactant from positive sera by incubation with calf thymus or guinea pig nuclei argue against the enzyme theory. Soluble DNA-protein complexes derived from calf thymus nuclei do not yield clots upon addition of hexamminecobaltic chloride, but do exhibit turbidities directly related to the amount of such complex present in solution. Since evidence from agglutination and precipitin studies indicates that globulin bound to nuclear extracts may well be a true antibody to such materials, it seems likely that formation of a soluble DNA-DLE serum complex prevents the polynucleotide from being precipitated in visible strands by cation. These observations resemble those of Lee(9), who used the agglutination of leukocytes by quinacrine as the phenomenon inhibited by DLE serum. The method described above deals with much more highly purified materials, has a more definite end-point, and seems of promise in assaying discrete serum fractions.

Summary. Normal sera do not inhibit the grossly visible clot formed by addition of hexamminecobaltic chloride to aqueous solutions of calf thymus DNA. The sera of approximately 66% of patients with DLE inhibit such clot formation. Prior treatment of DNA by active DNase also prevents clot formation, as does addition of histone or protamine to normal sera. The serum factor responsible for such inhibition is removed by prior incubation with whole calf thymus or guinea pig nuclei.

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Influence of Unbalanced Diets on Nitrogen Content and Enzymatic Activities of Hepatic Mitochondria of Rats. (25562)

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The aim of this work is to study whether an unbalanced dietary protein intake may cause changes in nitrogen content of the hepatic mitochondria. Previous observations(1) demonstrated that in normal rats fed protein deficient diet or fasted, some enzymatic activi-

TABLE I. Diet Composition.

	Normal balanced diet		Hypoprotein diet		Hyperprotein diet	
	per 100 g					
Casein	18	g	4	g	40	g
Rice starch	68		82		46	
Olive oil	10		10		10	
Salt mixture (Osborne & Mendel)	4		4		4	
Vit. D_2	62	units	62	units	62	units
" K	20	g	20	g	20	g
Thiamin	.4	mg	.4	mg	.4	mg
Lactoflavin	.8		.8		.8	
Pyridoxine	1.2		1.2		1.2	
Ca d-pantothenate	2		2		2	
Nicotamide	12		12		12	
P-aminobenzoic acid	120		120		120	
Biotin	20	g	20	g	20	g
Vit. B_{12}	4	μg	4	μg	4	μg
Folic acid	40		40		40	
Inositol	60	mg	60	mg	60	mg
Choline	120		120		120	

ties of the hepatic mitochondria/mg of mitochondrial nitrogen varied inversely with protein intake. These data led to the hypothesis that protein deficiency may cause a reduction of nitrogenous material in mitochondria and that consequently it may not be always advisable to relate enzymatic activity of mitochondria, to their nitrogen content, as is generally done. In our work mitochondria were counted to study the effect of fasting and of hypo- and hyperprotein diets on nitrogen content of each mitochondrion and on concentration of several enzymes/single mitochondrion and/mg of mitochondrial nitrogen.

Materials and methods. Male Wistar rats about 90 days old were fed 3 different food mixtures (Table I). All animals received a weekly supplement of 350 units of Vit. A. At end of experiment, the animals were fasted 12 hours and decapitated. The liver was quickly removed and the homogenate prepared at 0°. Mitochondria were separated from homogenate according to Schneider(2) in a solution of 0.25 M sucrose and 0.01 M ethylenediaminotetraacetic acid (EDTA), and washed 3 times in the same solution. The following activities were investigated: cytochrome-oxidase(3), succinic-oxidase(3), beta-hydroxybutyric-oxidase(3), ATPase(4). Nitrogen was determined by Nessler's reagent. Mitochondria

TABLE II. Nitrogen Content and Enzymatic Activities.

Diets	No. exp.	Nitrogen/mitochondrion, mg × 10^{-11}	Cytochrome oxidase: Mitochondrion × 10^{-8}		Cytochrome oxidase: mg N		Succinic oxidase (μl of O_2/hr): Mitochondrion × 10^{-8}		Succinic oxidase: mg N		Hydroxybutyric oxidase (μl of O_2/hr): Mitochondrion × 10^{-8}		Hydroxybutyric oxidase: mg N		ATPase (μmoles P/hr): Mitochondrion × 10^{-8}		ATPase: mg N	
1. Normal	15	3.10 ± .12	5.83 ± .35		2000 ± 122		2.50 ± .32		700 ± 53		2.69 ± .34		930 ± 69		6.0 ± 1.0		160 ± 13	
2. Hypoprotein	15	1.75 ± .08	4.50 ± .30		3200 ± 186		1.30 ± .20		1110 ± 124		1.16 ± .24		860 ± 65		4.0 ± .6		182 ± 19	
3. Fasting	13	.92 ± .04	2.87 ± .39		3100 ± 218		.80 ± .17		1300 ± 123		1.00 ± .24		1500 ± 155		3.0 ± .5		288 ± 33	
4. Hyperprotein	13	3.11 ± .11	10.20 ± .65		3320 ± 190		2.45 ± .50		720 ± 48		2.58 ± .4		950 ± 71		6.0 ± 1.5		160 ± 14	
Statistical significance																		
1-2		P <.05	P <.05		P <.001		P <.05		P <.05		P <.001		P >.05		P <.001		P >.05	
1-3		P <.001	P <.001		"		P <.001		P <.001		"		P <.001		"		P <.001	
1-4		P >.05	"		"		P >.05		P >.05		P >.05		P >.05					

were counted in a counting chamber using a phase-contrast microscope.

Results. Table II shows results. The hypoprotein diet caused marked reduction of nitrogen content without conspicuous decrease in enzymatic activities of single mitochondria. Fasting caused a very distinct decrease of nitrogen content with simultaneous decrease of enzymatic activities of single mitochondria. However, since decrease in enzymatic activities was smaller than decrease in nitrogen content, activity/mg of mitochondrial nitrogen appears to have increased. The hyperprotein diet did not cause significant changes in mitochondrial nitrogen content. On the other hand, it caused marked increase of cytochrome-oxidase activity/mitochondrion and/mg of mitochondrial nitrogen. Other enzymatic activities studied did not change significantly.

Discussion. It is obvious that under suitable experimental conditions the nitrogen content of hepatic mitochondria may undergo conspicuous variations and that by means of suitable protein deficient diets it is possible to reduce mitochondrial nitrogen content without reducing activity of certain enzymes significantly.

The results of these experiments suggest that hepatic mitochondria contain 2 nitrogen fractions with different functional characteristics: an enzymatically inert labile fraction and an enzymatically active one. Protracted fasting causes greater decrease of mitochondrial nitrogen and a much more pronounced fall of enzymatic activities, suggesting that, in this condition, there is significant loss of enzymatically active nitrogen. This appears to be bound more firmly to the mitochondrial structure than the enzymatically inactive fraction, which can be reduced by protein deficient diet. The increased cytochrome-oxidase activity observed following the hyperproteic diet suggests that the portion of the enzyme which under normal conditions is enzymatically inactive, may become activated. Our results further suggest that in experimental conditions able to change the nitrogen content of mitochondria, the data concerning enzymatic activities should be reported in terms of single mitochondria, rather than in terms of mg of nitrogen, as it is usually done.

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Blood Thrombocyte Levels after Administration of Equine Estrogens. (25563)

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Intravenous estrogens in uncontrolled studies have been reported beneficial in cases of hemorrhage of various types including neonatal(1), uterine(2,3), post dental extraction (4), nasal and post adenoidectomy(2,5), as well as rectal, gastric, and subarachnoid bleeding. However, the mechanism of reported hemostatic effect has not been adequately explained. In dogs, Johnson(6) found elevation of prothrombin and Ac-globulin as well as decrease of antithrombin following intravenous Premarin.* Increased levels of prothrombin and prothrombin-conversion accelerators have been found in late pregnancy(7). Thrombocytosis following estrogen injection was reported by Menger(2) but not seen by Jacobson(8) or Whittington(4). A sharp elevation of thrombocyte counts occurs just

* "Premarin," available from Ayerst Lab., consisting of a mixture of equine conjugated estrogens.