subsidence of the secondary aldosteronism, or may indicate a thyroid-adrenal interaction. A simpler explanation is that thyroidectomy effects a decrease in hepatic blood flow to this previously congested organ causing the ascites to form at a slower rate, and enabling gradual absorption of the accumulated fluid. Investigation of hepatic blood flow during the period of formation of ascites, and during recovery phase following thyroidectomy is now in progress.

Summary. Amelioration of experimental canine ascites produced by supradiaphragmatic caval constriction occurred in 7 of 8 dogs following total thyroidectomy. This improvement was accompanied by increase in sodium and water excretion. Dogs treated by a sham operation exhibited neither a natruresis nor relief of their ascites. Possible mechanisms to explain these results are discussed.

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Factors Affecting Liver Pyridine Nucleotide Concentration in Hyperthyroid Rats.* (24775)

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Severe hyperthyroidism affects concentration of tissue pyridine nucleotides and activities of certain pyridine nucleotide-dependent enzyme systems. Concentrations of pyridine nucleotides (PN)‡ in tissues of severely hyperthyroid rats are low according to several groups of investigators(1-3), but in one instance(4) PN concentrations above normal have been reported. A low rate of oxidation of D-β-hydroxybutrate by liver mitochondria from hyperthyroid rats has also been observed; however, the rate is increased markedly if DPN is added to the *in vitro* assay system(2). The rate of choline oxidation in livers of severely hyperthyroid rats is also low and this, too, is increased if a high level of DPN is added to the *in vitro* assay system (5). A variety of natural products and methyl donors, methionine and betaine, prolong survival of rats receiving high level of a thyroid-active substance(6,7). Pork, which is particularly effective in prolonging survival, also prevents to some extent the fall in rate of choline oxidation in livers of hyperthyroid rats(5). This investigation was undertaken to study effects of a number of dietary components, such as thyroid-active substances, protective factors, and PN precursors on liver

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‡ The following abbreviations will be used: PN, pyridine nucleostide(s); DPN, diphosphopyridine Nucleotide; TPN, triphosphopyridine nucleotide; TAP, adenosine, triphosphate.

PN concentration and to determine whether a relationship between liver PN concentration and survival time could be detected.

Methods. Male weanling rats§, weighing 40-50 g, were used. Rats were housed in individual suspended, screen-bottom cages; fed *ad lib.*, and weighed weekly during 19-21 day experimental period. Diets contained generous amounts of all known essential nutrients; 24% of protein, provided as either casein or pork; and 5% of corn oil. The carbohydrate was sucrose. Exact composition of diets and method of preparing the pork have been described(7). Supplements were included in diets at the expense of sucrose. In Exp. 2, a dietary level of 0.013% of sodium L-thyroxine was chosen to approximate that ingested by rats fed 0.4% of iodinated casein‖, on the assumption that iodinated casein contains 3.07% of thyroxine(8). Liver PN concentrations were determined by the method of Feigelson *et al.*(9). This method of analysis does not distinguish between DPN and TPN but rather measures their sum. Addition of sodium L-thyroxine to liver homogenates, to give a thyroxine concentration of 10^{-4}M, did not affect results of the assay for PN. Liver protein was determined by the biuret method of Mokrasch and McGilvery(10).

Results. Values for liver PN concentrations of rats fed diets containing either casein or pork, but no iodinated casein, were similar and were between 950-1050 μg PN/g of fresh liver (Table I). Values for rats fed similar diets but containing 0.4% of iodinated casein were lower. Liver PN concentration for rats fed the diet containing casein and iodinated casein was the lowest, about 77% of that for the euthyroid control group, a highly significant difference ($P<0.01$). The value for group that received pork and iodinated casein was intermediate. It was significantly lower than the value for the euthyroid control ($P<0.01$) but was significantly ($P<0.05$) above the value for the group receiving casein and iodinated casein.

Liver PN concentrations of rats, killed after only 14 days on experimental diets, were similar to those of rats killed after 21 days. Also, relative PN values were similar whether results were calculated/unit of liver protein or /unit fresh liver. The difference between the value for hyperthyroid rats and that for normal rats was actually slightly greater, when results were expressed in terms of liver protein because protein concentrations of livers from hyperthyroid rats were slightly higher than those of livers from euthyroid rats.

As different thyroid-active substances have been used to induce hyperthyroidism, the effects of several different thyroid-active materials on liver PN concentration were determined in Exp. 2. Degree of hyperthyroidism produced by each of the thyroid-active substances may not have been the same, so it may not be justifiable to compare directly liver PN concentrations of the different groups of hyperthyroid rats. Nevertheless, the results presented in Table I indicate that there

TABLE I. Effect of Diet and Age on Liver Pyridine Nucleotide Concentration of Hyperthyroid Rats.

Exp.	Diet	Rats per group	Total PN/g fresh liver, μg
1	Casein	6	1044 ± 28*
	Pork	6	1023 ± 21
	Casein + .4% I.C.†	6	796 ± 22
	Pork + "	6	869 ± 23
2	Casein	4	948 ± 31
	+ .4% I.C.	2	782 ± 9
	+ 2% desiccated thyroid‡	4	689 ± 19
	+ .013% Na L-thyroxine	5	767 ± 25
	+ Na L-thyroxine inj.§	7	702 ± 30
	+ .5% thiouracil	8	916 ± 16
	Idem + .4% I.C.	7	872 ± 26
3	Casein	6	1031 ± 18
	" + .4% I.C.	6	747 ± 16
	Idem + .04 mg % cyanocobalamin	6	763 ± 27
	" + .6% DL-methionine	7	784 ± 15
	" + 1% betaine·HCl	6	804 ± 20
	" + 3% choline	8	777 ± 10

* Mean ± stand. error of mean.
† Iodinated casein, Protamone, Cerophyl Laboratories, Kansas City, Mo.
‡ The Wilson Laboratories, Chicago, Ill.
§ 1 mg Na L-thyroxine/100 g body wt inj. intraperitoneally daily.

§ Holtzman Co., Medison, Wisc.

‖ Protamone, Cerophyl Laboratories, Kansas City, Mo.

TABLE II. Effect of Dietary Nicotinic Acid and Nicotinamide on Liver Pyridine Nucleotide Concentration of Hyperthyroid Rats.*

Diet	Total PN/g fresh liver, μg	
	No I.C.†	+ .4% I.C.
Casein	952 ± 54‡	736 ± 20
+ low vitamins§	913 ± 19	
+ .04% nicotinic acid		724 ± 32
+ .1% "	1407 ± 125	830 ± 33
+ .5% "	1424 ± 107	862 ± 30
+ .04% nicotinamide		855 ± 47
+ .1% "	1324 ± 40	858 ± 48
+ .5% "	1328 ± 121	816 ± 35

* 5-9 rats/group.

† Iodinated casein, Protamone, Cerophyl Laboratories, Kansas City, Mo.

‡ Mean ± stand. error of mean.

§ Water-soluble vitamin content of this diet reduced to 1/5 of casein basal diet.

was a marked decrease in liver PN concentration regardless of the method used to induce hyperthyroidism. Results for rats fed 0.5% of thiouracil show that hypothyroidism did not affect liver PN concentration, which agrees with results of Glock and McLean(3). When both thiouracil and iodinated casein were included in the diet, the decrease was not significant ($P>0.05$).

In Exp. 3, the effects of some compounds known to prolong survival of hyperthyroid rats were studied. Addition of an extra 0.04 mg % of cyanocobalamin to the 0.01 mg % already present in the diet, failed to increase liver PN concentration in the hyperthyroid rat. Also, liver PN concentrations of rats fed extra methionine, betaine, or a high level of choline were not significantly different ($P>0.05$) from those of the negative control group.

The effects on liver PN concentration of different levels of nicotinic acid and nicotinamide in diets of normal and hyperthyroid rats are shown in Table II. When amount of water-soluble vitamin mixture was decreased to one-fifth the amount usually fed, there was no significant effect on liver PN concentrations of euthyroid rats; but when rats were given an additional 0.1% of nicotinic acid (the casein basal diet contained 0.01% of nicotinic acid), there was a significant increase ($P<0.01$) in liver PN concentration. Raising the dietary level of nicotinic acid to 0.5% did not further increase liver PN concentrations. A similar increase was obtained when 0.1% or 0.5% of nicotinamide was added to the basal diet in place of nicotinic acid. Higher dietary levels of nicotinic acid (0.1% and 0.5%) and levels of 0.04%, and 0.1%, and 0.5% of nicotinamide significantly increased liver PN concentrations in hyperthyroid rats ($P<0.05$) but the values were well below those for normal rats. In hyperthyroid rats as in normal rats, 0.5% of either nicotinic acid or nicotinamide was no more effective in increasing liver PN concentrations than was the 0.1% level.

Since liver PN concentrations of hyperthyroid rats receiving high dietary levels of nicotinic acid or nicotinamide were approximately the same as those of hyperthyroid rats fed pork, the effects of high dietary levels of these vitamins on survival of hyperthyroid rats was determined. Average survival times of groups of rats fed the basal diet containing casein and 0.4% of iodinated casein unsupplemented, or supplemented with 0.1% or 0.5% of nicotinic acid or with 0.1% or 0.5% of nicotinamide were as follows: no supplement, 29 days; 0.1% of nicotinic acid, 28 days; 0.5% of nicotinic acid, 29 days; 0.1% of nicotinamide, 27 days; and 0.5% of nicotinamide, 20 days. High dietary levels of nicotinic acid or nicotinamide did not prolong survival of hyperthyroid rats. In fact the higher level of nicotinamide seemed to decrease survival time of hyperthyroid rats further, while nicotinic acid had no such effect. In normal animals, nicotinamide is more toxic than nicotinic acid(11).

Discussion. No direct relationship between liver PN concentrations and survival time of hyperthyroid rats was seen. Dietary pork, which markedly prolongs survival of hyperthyroid rats(6), increased liver PN concentration, but not to that of normal controls. Vit. B_{12}, methionine, betaine, and a high level of choline also exert a protective effect in hyperthyroid animals(7,8,12-15), but they did not cause an increase in liver PN concentration of hyperthyroid rats. If, however, the protective action of methionine and betaine can be explained by the fact that these compounds supply methyl groups so that the rat does not have to depend upon the DPN-re-

quiring choline oxidase system, then their protective effect might be independent of liver PN concentration. Nevertheless, ingestion of a high dietary level of nicotinic acid or nicotinamide did not prolong survival of hyperthyroid rats, although it did cause an increase in liver PN concentration. Even highest levels of these vitamins did not completely restore PN concentration in livers of hyperthyroid rats to normal as observed by Katzenelbogen *et al.*,(1). The failure of high dietary levels of nicotinic acid or nicotinamide to prolong survival of hyperthyroid rats could be explained if the high dietary levels of these vitamins increased the demand for methyl groups and thus exhausted any additional supply of methyl groups that might have been formed through increased choline oxidation. An increased need for methyl groups has been observed when high levels of nicotinamide are fed(11).

Liver PN concentrations of hyperthyroid rats fed either pork or high levels of nicotinic acid or nicotinamide were similar; however, in normal rats, feeding of pork did not increase liver PN concentrations (Exp. 1), whereas ingestion of high levels of nicotinic acid or nicotinamide caused PN concentrations in livers of normal rats to rise 40-50% (Table II). Furthermore, pork muscle contains only 4 mg of nicotinic acid/100 g of fresh tissue(16), thus supplying only an additional 0.007% of nicotinic acid in the diet. Therefore, the ability of pork to increase liver PN concentrations of hyperthyroid rats is apparently not attributable to the provision of these vitamins by pork.

The results of these experiments confirm those of several investigators(1-3) who found a decrease in liver PN concentration in hyperthyroid rats but are contrary to those of Galeone *et al.*,(4). who fed and injected thyroxine to induce hyperthyroidism; Glock and McLean(3) injected thyroxine; and the others(1,2) fed desiccated thyroid. Since, in our study, there was a marked decrease in liver PN concentration in rats made hyperthyroid by the 4 different treatments, the discrepancy between the 2 sets of observations cannot be attributed to use of different thyroid-active substances. It is also improbable that the decreased liver PN concentrations of hyperthyroid rats are a result of the weight difference between hyperthyroid and normal rats of the same age. Normal rats weigh as much after 14 days on experiment as hyperthyroid rats do after 21 days, yet there was a marked difference between liver PN concentrations of the 2 groups when they were equal in weight or in age.

Summary. No relationship was observed between survival time and liver pyridine nucleotide concentration of hyperthyroid rats. Pork, which prolongs survival, and nicotinic acid and nicotinamide, which do not prolong survival, partially prevented the fall in liver pyridine nucleotide concentration caused by thyroid-active substances. Methionine and betaine, which prolong survival, did not cause an increase in liver pyridine nucleotide concentration.

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