tissue following partial hepatectomy may have contributed to the increase of phagocytosis also in our experiments, but since we measured phagocytosis 24 hours after intraperitoneal injection, it is less likely that this was the decisive factor. Simultaneously with preliminary report of our results(5), Leong and associates(6) presented data obtained by isolated rat liver preparations perfused with radioactive colloidal chromic phosphate. In this study the authors found that 48 hours after hepatectomy phagocytic activity of liver had doubled over that of controls. Since these results were obtained with controlled rates of perfusion *in vitro,* they do not support the assumption that increased rate of blood flow was the main factor in elevating hepatic phagocytosis after partial hepatectomy. Abercrombie and Harkness(7) observed that regeneration of littoral cells proceeded at a faster rate than that of parenchymal cells between 2 and 7 days after partial hepatectomy. Similarly, Aterman(8) noted an increase in sinusoidal cells as early as 3 hours after partial hepatectomy It is also of interest to refer to observations by Haven *et al.*(9) on increased immune responses in rats after partial hepatectomy; when injections of sheep red cells were given immediately after hepatectomy, these animals showed significantly higher hemolysin titers than did controls.

Summary. 1. Uptake of Au^{198} in liver and spleen was compared after hepatectomy or sham operation was carried out in Lewis and Wistar rats. 2. Female rats showed higher uptake of radiogold in liver and spleen than did males. 3. Three and 8 days after hepatectomy, male Lewis rats, and 3 days after hepatectomy male Wistar rats, showed greater uptake of radiogold in liver and spleen than did sham-operated controls. Similar but less pronounced differences were found in female Lewis rats. 4. Fifteen or 16 days after hepatectomy male and female Lewis rats showed no significant differences in hepatic or splenic uptake of radiocolloid, while in male Wistar rats splenic but not hepatic uptake was still increased. 5. Increased spleen/body weight ratios were observed in hepatectomized rats.

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The Free Myo-Inositol of Thyroid Tissue.* (24693)

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During a study of phospholipid metabolism of sheep thyroid tissue(1-3), abundant quantities of a water-soluble compound were noted which exhibited chromatographic mobility and staining characteristics of free myo-inositol. In addition, in lipid extracts of sheep thyroid tissue, an inositol-containing phosphoglyceride was demonstrated. *In vitro*(2), as well as *in vivo*(3), the P^{32} turnover in this phosphoinositide was 2 to 10 times more rapid

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than that of any other lipid component. Comparable earlier observations are limited. In 1946, Meyer, on the basis of C and H analysis and melting-point determinations, identified as inositol the crystals which appeared in alcoholic extract of commercial desiccated thyroid powder(4). He estimated that the "inositol" accounted for 0.3% of the powder and cited Tambach's original report in 1896 of "the presence of inositol in aqueous extracts obtained from thyroids in manufacturing process"(5). The present studies were designed to provide more definitive information about the thyroid content of inositol. Levels of free myo-inositol in the thyroid gland of a variety of species have been determined by specific microbiological assay(6). Preparation and extraction of tissues were designed to minimize the breakdown of inositol-containing phospholipids, proteins, and water-soluble phosphate esters.

Methods. Specimens of human thyroid were obtained at surgery of patients with Graves' Disease or non-toxic nodular goiter. Sheep thyroid glands were obtained immediately following exsanguination of animals at abattoir. Rabbit, rat, and guinea pig glands were rapidly removed from animals which had been anesthetized with ether or stunned by blow on head. The excised tissues were placed directly on powdered solid CO_2 for quick-freezing and then extracted by homogenization with iced 5% perchloric acid (PCA) in Potter-Elvehjem ground-glass homogenizers. The chilled acid extracts were separated from insoluble residues by centrifugation and filtration through Whatman #42 filter paper. PCA in aliquots of supernatant was precipitated by neutralization with 5 N KOH at 0°C. Whenever possible, specimens of heparinized blood were obtained simultaneously and aqueous extracts of blood and plasma were prepared by precipitating plasma proteins and lipids with PCA. Neutralized filtrates were diluted so that 1 to 5 μg inositol were contained in 2.5 ml. This volume was combined with equal quantity of medium described by Campling and Nixon(6) and, after autoclaving, was inoculated with Kloeckera Brevis yeast and grown for 3 days at 25°C. Yeast growth was assessed by turbidimetric assay. All samples were analyzed in triplicate and related to duplicate standard curves which were obtained concurrently.

TABLE I. Concentration of Free Myo-Inositol in Thyroid Glands and Plasma of Various Species.

Species	Free myo-inositol* Thyroid (mg/100 g)†		Plasma (mg/100 ml)	
Human	126	76–166 (4)	.45	.43– .46 (4)
Rabbit	200	126–350 (4)	.91	.62–1.20 (2)
Rat	135	56–255 (12)	1.22	.90–1.61 (5)
Guinea pig	372	215–550 (8)	1.70	.92–3.67 (4)
Sheep	355	276–432 (4)		

* Values represent mean and range. No. in parentheses represent No. of individuals in each group.
† Values derived on basis of wet wt of fresh thyroid tissue.

Results are listed in Table I. The Kloeckera Brevis yeast assay measures only free myo-inositol. By this criterion, the mean thyroid/plasma concentration differentials for free myo-inositol were greater than 100 in every species examined. Hydrolysis of aqueous extracts in 5 N HCl for 20 hours at 105°C did not increase inositol concentration thus indicating that the thyroid content of inositol phosphates is inappreciable. Enhancement of "free myo-inositol" due to liberation of inositol by hydrolysis of inositol esters during preparative manipulations does not seem likely. Similar concentrations of free myo-inositol were found in control studies with guinea pig thyroid wherein a) one lobe was excised immediately and frozen on solid CO_2 (mean 445 mg/100 g) and b) the second lobe was left *in situ* for 4 minutes prior to excision (mean 465 mg/100 g). Moreover, levels of free myo-inositol were not significantly different in thyroids of anesthetized and "stunned" animals thus precluding the likelihood of an anesthesia artifact. Finally, paper chromatography of aqueous extracts of guinea pig thyroid in ethanol/NH_3, isopropanol/acetic acid, and cellosolve/butanol/ethanol/NH_3 solvents confirmed the presence of considerable amounts of inositol in the gland.

Discussion. The thyroid gland of every species examined contained abundant quantities of free myo-inositol. On the basis of known compositional characteristics of sheep

thyroid tissue, some quantitative dimensions may be assigned to the free inositol in this species. Since average dry weight of sheep thyroid gland constitutes 20.3 ± 3% of wet weight(7), the average inositol level of 355 mg/100 g observed in the present study would account for 1.7% of dry weight of ovine thyroid. In terms of ultimate disposition, only a small fraction of this free inositol need be necessary for incorporation into phosphoinositide. For example, lipid phosphorus in the sheep thyroid averages 191.5 μg P/g wet weight and 9% of this is present in the phosphoinositide(2). Thus, assuming that thyroid phosphoinositide is a monophosphoinositide, its inositol content maximally would equal only about 0.3% of free inositol.

For the moment, the physiological significance of free myo-inositol in thyroid tissue is unclear, nor has it been determined whether the thyroid can concentrate or synthesize inositol. Eagle has demonstrated the role of myo-inositol as an essential growth factor in tissue culture(8). Tissue analyses in rats, guinea pigs and rabbits (to be published elsewhere), have indicated that free myo-inositol is significantly more abundant in thyroids of these species than in salivary gland, heart, liver, spleen, kidney cortex, adrenal, pancreas, testicle, ovary, lung, mammary gland and diaphragm. Screening studies of multiple tissues have indicated some correlation between levels of free inositol and protein elaboration. Thus, conceivably, the abundant myo-inositol within the thyroid could be linked to the active intrathyroidal accumulation of amino acids (or other anions such as iodide), protein synthesis, or secretion of thyronines. Alternatively, in view of known conversion of inositol to glucuronic acid(9,10) some of the free myo-inositol within the thyroid might be implicated in active formation of intrathyroidal mucopolysaccharides(11). Experimental efforts to assess these and other possibilities and to correlate inositol levels with thyroid activity are in progress.

Summary. Bioassay with Kloeckera Brevis yeast has been employed to measure content of free myo-inositol in human, rat, rabbit, sheep and guinea pig thyroid glands. In all species, thyroid/plasma concentration differentials for free myo-inositol were greater than 100. Chromatographic documentation has also been secured, and the possible significance of the abundant intrathyroidal myo-inositol has been discussed.

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Propagation of Canine Distemper (CD) Virus in Tissue Culture. (24694)

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Repeated attempts by the authors to grow CD virus (canine distemper virus) of dog or ferret origin in tissues of those 2 species yielded essentially negative results. Presumably experience of others has been similar; no report of a successful attempt has come to our attention, among numerous publications in the past 5 years on tissue-culture propagation of many different animal viruses. This communication describes the successful propaga-