not possible to say what relationship may exist between the follicular conjunctivitis which we observed and other ovine eye lesions that were attributed to unidentified rickettsial agents(15). It should not be surprising if PLT agents will be isolated from the latter conditions as was suggested by Dickinson and Cooper(16). However, we cannot see any possibility that the unidentified infectious agent incriminated by Livingston *et al*(17) as causing keratoconjunctivitis in sheep, could be a PLT or rickettsial organism.

Summary. Psittacosis - lymphogranuloma-trachoma agents were isolated from conjunctival scrapings from 9 of 17 sheep representing 3 different flocks afflicted to 90% with follicular conjunctivitis. The PLT agent isolated from follicular conjuctivitis was specifically related to those causing polyarthritis. Ten to 25% of the sheep with eye lesions had polyarthritic signs and the PLT agents were isolated from the blood of 6 of the 17 sheep tested. Parenteral inoculation resulted in follicular conjunctivitis and polyarthritis.

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Comparative Activties of Aspartate Transcarbamylase in Various Tissues of the Rat.* (32224)

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In recent years considerable attention has been given the enzyme, aspartate transcarbamylase (ATC), which catalyzes the first step unique to the biosynthesis of pyrimidines. This enzyme is found widely distributed throughout the plant and animal kingdom including bacteria(1,2), fungi(3), higher plants(2,4), birds(5), and mammals(5,6,7). Of primary concern have been investigations of the possible interactions between pyrimidine end-products and ATC in the regulation of pyrimidine anabolism(6,8,9,10,11). As yet no common denominator for control of pyrimidine biosynthesis is known for all species. Although ATC has been partially purified from certain mammalian tissues and characterized with regard to kinetics and substrate specificity, limited information is available concerning its tissue distributon. However, some localzation studies of this enzyme have been done in certain plants(4) and to a more limited extent in rat tissues(5).

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The objective of this study was to determine the relative activities of ATC in a variety of tissues in the rat and to estimate the variation of tissue activities within a group of animals.

Materials and methods. The method of assay for ATC was a modification of an existing method(5) which utilizes carbamyl phosphate-C^{14} as a substrate. This compound was prepared according to Jones *et al*(12). Following two recrystallizations from cold ethanol the compound was assayed radiochemically and its identity verified by its infrared spectrum.

Preliminary studies to determine the optimal conditions for ATC assay were performed utilizing centrifugally clarified supernatants of Holtzman rat liver homogenates. All assays were done in duplicate. To determine the optimum pH, a "universal" buffer system was employed. The stock buffer solution consisted of 0.03 M each of diethylbarbituric acid, citric acid, boric acid and KH_2PO_4. This stock solution was divided into equal quantities and each portion was titrated to the desired pH over the range of 6 to 11 with measured amounts of 2 N NaOH. To maintain approximate equivalency in ionic strength throughout, appropriate quantities of 2 M NaCl were added to each buffer solution to equal the difference between the quantity of titrant required to adjust to pH 11, and the quantity of titrant required for each succeeding buffer. Each assay tube contained a total volume of 1.0 ml and consisted of 0.1 ml enzyme, 0.1 ml 0.05 M carbamyl phosphate-C^{14}, 0.2 ml 0.04 M aspartate, and 0.6 ml of the appropriate buffer. The pH of each assay mixture was measured prior to and at the end of the incubation period. Each sample was incubated 30 minutes at 37°C along with the appropriate blanks to which enzyme was added after the reactions were stopped with addition of 0.5 ml 1.5 N trichloroacetic acid. To decompose and eliminate the unreacted carbamyl phosphate-C^{14} as CO_2-C^{14} the samples were immersed in a boiling water bath for 10 minutes under the hood. The product of the enzymic reaction, carbamyl aspartate-C^{14}, is stable under these conditions and is therefore retained in solution. Following centrifugation, 0.1 ml of each clarified supernatant was mixed with 7 ml scintillator solution and the radioactivity was measured at 0°C in a Packard Tri-Carb liquid scintillation system at 45% efficiency. The scintillation solution contained 3.5 g 2,5-diphenyloxazole, 3.55 mg 1,4-bis-2(4-methyl-5-phenyloxazolyl) benzene, 100 ml anisole, 100 ml dimethoxyethane, and 500 ml p-dioxane. The observed optimum activity was at pH 9.2. Utilizing 0.2 M Tris buffer, tris(hydroxymethyl)aminomethane, (pH 9.2) the aspartate optimum was found to be 8 mM, utilizing 9 concentrations from 0.2 to 24 mM aspartate and 5 mM carbamyl phosphate-C^{14}. The optimum carbamyl phosphate concentration was established at 12 mM from data of 8 different concentrations of this substrate from 0.2 to 24 mM.

Male Holtzman rats, 3-4 months old, were anesthetized with sodium diethylbarbital and exsanguinated by cardiac puncture. The blood was heparinized and the cells and plasma separated by centrifugation. Certain whole organs and samples of various other tissues were excised, washed thoroughly in ice-cold isotonic saline, and stored at −20°C until the assays could be performed. Prior to assay each tissue sample was thawed and homogenized in 0.2 M Tris (pH 9.2) containing 2.0 mM mercaptoethanol and 1.0 mM EDTA with a Potter-Elvehjem all-glass homogenizer. The protein content of each homogenate was estimated by the technic of Lowry *et al*(13) utilizing a commercial standard ("Lab-Trol," Dade Reagents, Inc.) for calibration. Each assay contained a total volume of 1.0 ml and consisted of 0.1 ml enzyme, 0.1 ml 0.12 M carbamyl phosphate-C^{14}, 0.2 ml 0.04 M aspartate, and 0.6 ml 0.2 M Tris buffer containing mercaptoethanol and EDTA. The pH of each sample was measured and adjusted to pH 9.2 when necessary prior to incubation at 37°C for 30 minutes. Appropriate blanks were prepared for each sample as described above.

Results. The results of ATC assays in various rat tissues as presented in Table I are expressed as the mean specific activities accompanied by the standard error estimates. A unit of enzyme activity is expressed as

TABLE I. Tissue Activities (Means ± S.E. of Means) of Aspartate Transcarbamylase in the Holtzman Rat. Enzyme activities expressed in mμmoles carbamyl aspartate synthesized per mg of tissue protein per 30 minutes.

Tissue	Avg specific activity
Testis	332 ± 31.8 (10)
Spleen	265 ± 22.1 (10)
Intestinal mucosa	211 ± 25.4 (10)
Bone marrow	179 ± 40.3 (10)
Small intestine	175 ± 43.0 (8)
Liver	126 ± 12.6 (10)
Lung	114 ± 10.0 (10)
Kidney	75 ± 5.6 (10)
Brain	59 ± 5.9 (10)
Pancreas	35 ± 17.6 (9)
Heart	24 ± 3.8 (10)
Skeletal muscle	18 ± 3.8 (10)
Erythrocytes	17 ± 4.2 (10)
Plasma	3 ± 1.0 (10)

Numbers in parentheses indicate No. of animals.

millimicromoles of carbamyl aspartate synthesized per mg of tissue protein per 30 minutes. These results are tabulated in decreasing order of enzyme activity. The aspartate transcarbamylase activity of testis was found to be 332 ± 31.8 units (mean ± S.E.M.). This tissue contained the highest ATC activity of those examined. Other tissues found to contain high levels of ATC included spleen, intestinal mucosa and bone marrow. Those tissues of intermediate ATC activity included liver, lung, kidney, and brain. Other tissues examined contained comparatively low levels of this enzyme. Blood plasma was essentially devoid of activity.

Discussion. In attempting to correlate the aspartate transcarbamylase activity with nucleic acid synthesis, the data of Table I may be compared to the mitotic index of various tissues. Leblond and Walker(14) classified adult organs into 3 categories according to mitotic activity. These are (1) organs without mitotic division and thus no cell renewal: the central nervous system; (2) organs with low mitotic rate and low cell renewal which include liver, pancreas, kidney and vascular endothelia; and (3) those organs that exhibit frequent mitotic division and have cell renewal. This latter group includes the digestive, respiratory and genital systems and the blood forming organs. The organs of Group 3 examined for ATC activity included testis, spleen, small intestine, intestinal mucosa, bone marrow and lung. All of these tissues except lung were relatively high in enzymic activity. Of those organs exhibiting low mitotic activity (Group 2) pancreas, kidney and liver had moderate ATC levels. The moderately high ATC activity of brain obviously does not reflect cell division in the central nervous system but may be an indicator of *de novo* RNA synthesis.

Summary. Aspartate transcarbamylase activity was measured in various tissues of Holtzman rats. The distribution of this enzyme is correlated with mitotic activity in all tissues except brain.

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