vents described here and previously(4,5,6) are used, the leucine and isoleucine appear as a separate spot above the phenylalanine area (Fig. 1, 2). In addition, the specific phenylalanine reaction described here eliminates contaminating amino acids as they are washed away or extracted, leaving only the phenylalanine for quantitative measurements (Fig. 1, 2, 3).

Van de Heuvel and Wadman(7) described a paper chromatographic method for determining phenylalanine in serum in which the chromatographic process was performed at 37°C. They removed salt prior to chromatography and used a ninhydrin reagent for staining the amino acids. In our method no desalting is required and the bicarbonate treatment following ninhydrin is selective for phenylalanine.

Recently the paper chromatographic technique of Hudson, Dickinson and Ireland(8) for detection of phenylketonuria has come to our attention. They report that with the solvent system used it is possible to separate phenylalanine completely from valine and the leucines.

Summary. A specific reaction for phenylalanine has been applied after chromatographic separation of amino acids from urine, serum and blood specimens of phenylketonuric patients. This reaction is demonstrated by means of photographs of the actual chromatograms obtained.

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Flow Rate and Composition of Thoracic Duct Lymph With and Without Cisterna Chyle Ligation. (29077)

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It was suggested(1) that one method by which anti-inflammatory agents may act to reduce induced pleural edema may be by increasing lymphatic drainage. In attempting to determine the role of lymphatic drainage it was found that little information concerning the normal flow rate and composition from this area in rats was available(2). In view of the above it was thought that whether or not lymphatic drainage is associated with the reduction of pleural edema, some information on normal rats would be of value.

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The thoracic duct is known to be the final collecting pathway in many animals(3), receiving lymph from the lower extremities, peritoneal cavity and contents, left upper extremity and the left side of the head and neck. The right lymphatic duct drains the right extremity and the right side of the head and neck. Although the thoracic cage is drained mainly *via* the right lymph channel (4), we have shown that T-1824 dye appears in the thoracic duct within 20-30 seconds after injection into the left pleural space.

It was also of interest to determine contri-

bution of the cisterna chyle lymph to the thoracic duct. The composition of the lymph in the cisterna chyle and its flow rate have been previously shown(5).

Materials and methods. Male albino rats weighing 350-390 g were used in this study. To visualize the thoracic duct and the cisterna chyle, ½ cc of olive oil was given *via* stomach tube. The oil could be found in the thoracic duct within 10-20 minutes after introduction and was cleared within 3 hours. In all cases the oil was cleared prior to opening into the thoracic duct.

The rats were anesthetized with 42 mg/kg of sodium pentobarbital. The anesthesia was kept as light as possible. A gross comparison was made in the rate and depth of respiration before and after anesthesia(6).

An incision was made over the mid line of the clavicle exposing the upper fibers of the pectoralis muscle and approximately ½ the length of the sternomastoid muscle. It is necessary to cut the upper fibers of the pectoralis and the sterno-clavicular attachment of the sternomastoid muscle. The clavicle is separated from its sternal attachment and retracted away. This allows good access to the subclavian vein and the thoracic duct found entering it in the medial ⅓, deep surface of the vein. From this point all work must be done under magnification.

Just prior to entering the vein the thoracic duct receives a lymph vessel from the cephalic region. The junction of these two vessels causes a marked increase in the diameter of the duct. At this point, entrance is made into the duct.

Either of 2 procedures may now be followed: 1. Direct cannulation with polyethylene tubing or 2. micropipetting of the fluids in the duct. Both methods were used and essentially no difference was found in the flow rates measured. If the direct cannulation is to be tried, approximately 3-4 mm of the thoracic duct must be freed from its adherence to the vein. This allows a suture to be placed around the duct holding the cannula in place. A small bulge should be made in the polyethylene tube using a heated wire loop. A No. 10 polyethylene tube (I. D. .011-O. D. .024) may be used as the cannula. The opening into the duct can be made with ophthalmic scissors.

Using the second method the cut is made without freeing the duct. The edge of the cut can be held with a pair of very fine forceps. It is possible to see the fluid in the duct and remove it by means of a pipette.

If the cisterna chyle was to be ligated it was located prior to opening into the duct. A loose suture was placed around the cisterna chyle allowing the ends of the suture to remain long. If care is exercised there is no notable difference in lymph flow rate due to trauma to the peritoneal viscera. This was demonstrated by collecing lymph from the duct prior to and after abdominal surgery. The abdominal cavity was closed with clamps.

After the thoracic duct was opened the rate of flow was determined. It usually required approximately 30 minutes to collect enough lymph for analysis. Two animals were followed for 2 hours to demonstrate that there was no change in the flow rate over this time period. The abdominal cavity was carefully reopened and the cisterna chyle was ligated. A new flow rate and chemical composition were determined.

The lymph fluid was analyzed for total protein using the micro-Kjeldahl method. The relative amounts of albumin and globulin were determined by paper electrophoresis.

Results and discussion. The average flow rate of thoracic duct lymph per kilogram body weight was found to be 2.50 cc/hour. This compares favorably to the 2.00 cc/hour flow found by Reinhardt(2) using 200-250 g rats.

Nix(5) found the average flow rate of lymph from the cannulated cisterna chyle to be 1.86 cc/hr/kg. Nix used rats in the same weight range as Reinhardt. If we subtract 1.86 cc/hr/kg from the control 2.00 cc/hr/kg the resulting 0.14 cc/hr/kg should be approximately the flow rate of lymph in the thoracic duct after ligation of the cisterna chyle. The value obtained in this project was 0.266 cc/hr/kg (Table II).

Nix also found the average A/G ratio for

lymph from the cisterna chyle to be 2.57. This ratio represents a high percentage of albumin. It was found that our samples of control thoracic duct lymph had an average A/G ratio of 1.03 (Table II). This change in A/G ratio could be explained by either a loss of albumin or a rise in globulin concentration in the lymph while passing to the point of cannulation. The lymph obtained after ligation of the cisterna chyle had an average A/G ratio of 0.476 and a percent globulin of 69.3 (Table II). The average protein concentration of the cisterna chyle lymph was found by Nix to be 3.06 ± 0.2 g% while that in the thoracic duct was 3.89 ± .70 g% (Table I). This indicates that protein is added to the lymph in the thoracic cage area.

Five cc of saline was injected into the left pleural space of a rat *via* a 27-g. needle. After 10 minutes the animal was sacrificed and the fluid in the space was collected for analysis. The results given are the averages of 5 animals (Table III). Protein concentration was .105 g% and total protein was 5.04 mg. In all cases paper electrophoresis gave a strong γ-globulin and a lighter β-globulin band. In no case was there an albumin band visible. The γ-globulin band made up approximately 70% of the bands and the β-fraction approximately 30%. This further substantiates the view that the thoracic components contain relatively high concentrations of globulin.

TABLE I. Flow Rate and Protein Composition of Thoracic Duct Lymph.

Rat No.	A/G	Flow cc hr^{-1} kg^{-1}	Protein conc (g %)	Protein (g) hr^{-1} kg^{-1}
1	1.29	2.75	3.75	.104
2	.880	1.36	2.69	.087
3	1.20	2.93	4.71	.138
4	1.51	2.06	5.24	.109
5	1.14	3.10	4.37	.132
6	.670	2.06	3.88	.132
7	.670	2.75	2.75	.075
8	1.43	2.98	3.76	.112
Avg	1.10	2.50	3.89	.105

TABLE II. Comparison of Flow Rate and Protein Composition of Thoracic Duct Lymph Before and After Ligation of the Cisterna Chyle.

Rat No.	A/G	Flow cc hr^{-1} kg^{-1}	Protein conc (g %)	Protein (g) hr^{-1} kg^{-1}
2 T*	.880	1.36	2.69	.037
2 C	.480	.172	1.98	.0034
5 T	1.14	3.10	4.37	.132
5 C	.234	.344	2.78	.0086
7 T	.670	2.75	2.75	.075
7 C	.320	.287	1.84	.0052
8 T	1.43	2.87	3.76	.112
8 C	.870	.287	1.96	.0057
Avg T	1.03	2.49	3.39	.089
Avg C	.476	.266	2.14	.0057
% change C vs T				
	% red.	% red.	% red.	% red.
	53.8	89.4	36.9	93.5

* T = thoracic duct lymph. C = thoracic duct lymph after ligation of the cisterna chyle.

TABLE III. Protein Concentration and Albumin Globulin Composition of Saline Recovered from the Pleural Space of Rats.

Rat No. 1	Protein conc (g %)	cc of injected saline recovered	Total protein (mg)	% G	% β	% γ
1	.094	4.9	4.61	100	32	68
2	.087	5.1	4.43	100	43	57
3	.125	4.7	5.87	100	25	75
4	.107	4.7	5.03	100	21	79
5	.113	4.8	5.42	100	31	69
Avg	.105	4.8	5.04	100	30	70

The present paper does not attempt to determine if globulins tend to aggregate in the pleural tissues or if the globulins are actually formed in these tissues. Having large quantities of globulins, especially γ-globulin, may be a defensive mechanism against infectious invasion.

Summary. The flow rate and composition of lymph from the thoracic duct of rats were obtained before and after ligation of the cisterna chyle. Changes in flow rate after ligation of the cisterna chyle show that approximately 90% of the lymph in the thoracic duct is derived from the peritoneal area and lower extremities. Changes in the composition of the lymph indicate that the lymph from the thoracic area is high in globulin material.

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Effect of Various Substrates on Oxygen Uptake of Rat Cerebral Cortex Slices.* (29078)

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The well-known association of liver disease and coma implies that adequate liver function is required for normal brain metabolism. The usual explanation for this association has been that the liver is necessary to inactivate some substance which is probably absorbed from the gastrointestinal tract and is toxic to brain. Indeed, the demonstration that administration of ammonium salts or a precursor of ammonia induced a syndrome indistinguishable from spontaneous impending hepatic coma in certain patients with liver disease(1) lent support to this theory. The role of ammonia in development of spontaneous hepatic coma, however, has not been clarified.

Normal brain metabolism may depend on liver function in another way, by requiring some nutrient which is synthesized only or predominantly in the liver. In support of this theory, Geiger *et al*(2) reported that glucose uptake of perfused cat brain could be restored for a short time by adding fresh cat liver extract or cat blood to the perfusate and for a longer time by including the liver in the perfusion system. Geiger and Yamasaki(3) subsequently reported that this effect on glucose uptake could be produced by adding cytidine and uridine to the perfusate. Similarly, Lang, Goldstein, and Levine(4) showed that hepatectomy in dogs markedly decreased utilization of glucose by peripheral tissues, an effect which was independent of insulin, and postulated that the liver released a "humoral" agent necessary for normal peripheral glucose consumption.

In the present study an attempt was made to find a substance synthesized by the liver which might be required for normal brain metabolism. To do this, the effect of various known substrates which are made in the liver and might fulfill this role on oxygen uptake of surviving rat cerebral cortex slices was studied.

Materials and methods. Sherman rats weighing about 100 g each were used. The animals were stunned by a blow on the back of the head and exsanguinated; the brains were removed within a few minutes and placed in cold buffer of composition described below. Hemorrhagic brains were not used. Slices from each brain were cut free-hand from the cerebral cortex grey matter and placed into 3 manometric flasks, the first serving as a control and the other 2 containing different concentrations of the substrate to be tested. The buffer volume in each flask was 3.0 ml. The standard "direct" Warburg technique(5) was employed at 37.0°C using 9 test flasks (3 brains) and 2 thermo-barometer flasks for each run. The time elapsed between sacrifice of the rat and first manometric reading was about 60-75 minutes, and manometric readings were taken at 30-minute intervals for 5 hours. The brain slices were then removed, rinsed in distilled water, dried at 110°C for about 18 hours, and

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