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Distribution of Proteoses and Peptones After Intravenous Injection.

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This investigation was concerned with the distribution of proteoses and peptones in the mouse after intravenous injection. The metabolism and distribution of amino acids labeled with deuterium and N^{15} after oral and parenteral administration have been studied by Schoenheimer, Rittenberg, *et al.*[1] Freund and coworkers[2] determined the changes in the composition of non-protein nitrogen after intravenous injection of peptones and proteoses. They concluded that the peptones were absorbed into the intestine. Fine *et al.*[3] injected proteins containing radioactive elements (Br or S) into animals in shock. The rate of disappearance of radioactivity varied with the protein depending on the method by which the radioactive element was introduced.

In this work a pepsin digest of yeast containing N^{15}, uniformly distributed, was used. The distribution of the injected material was determined from the nitrogen and N^{15} analyses.

Materials. Yeast (*Saccharomyces cerevisiæ*) was grown in a synthetic medium (Frey* *et al.*[4]) in which the only source of nitrogen was ammonium chloride or sulphate containing 10 or 30% N^{15}. After 24 hours at 30°C with slow, continuous, and mild shaking, the yeast was centrifuged, washed repeatedly with water, acetone, and ether and finally dried in vacuum over sulphuric acid. Meisenheimer[5] has shown that 7.5 to 9.6% of yeast nitrogen is purine nitrogen and identified 15 amino acids which accounted for another 74% of the nitrogen. The presence of additional amino acids in yeast protein has been demonstrated more recently.[6]

The dried yeast was suspended in water and digested at pH 2 and at 40°C with a concentrated pepsin solution for two hours. The digest was neutralized and centrifuged. The supernatant was used for the injections. The insoluble residue contained carbohydrates and 16 to 18% of the original nitrogen. Peptones and proteoses were determined in the supernatant liquid as follows: 10 ml of the material, containing 0.2% nitrogen and 10% sodium sulfate, was acidified with acetic acid to pH 5 and one ml of a freshly prepared 10% tannic acid solution added. The solution was mixed thoroughly, kept for 24 hours at 0°C, and centrifuged at 0°C. The nitrogen content of the supernatant solution was determined. On the average 62% of the total nitrogen was precipitated by tannic acid, which compares favorably with the results of Wasteneys and Borsook[7] on pure peptone solutions. It may be assumed that at least 62% of the nitrogen in the supernatant consisted of peptones and proteoses.

Experimental. The solution was injected into the tail veins of fasting male albino mice weighing from 20 to 25 g. The peptone used in mice No. 1 and No. 3 of Table I contained 9.03% N^{15}, in all others 27.0% N^{15}.

The mice were killed by decapitation 10, 30,

1 Schoenheimer, R., and Rittenberg, D., *Physiol. Rev.*, 1940, **20**, 218; Rittenberg, D., *Cold Spring Harb. Symp.*, 1941, **9**, 283; Schoenheimer, R., *The Dynamic State of Body Constituents*, Harvard Univ. Press, 1942.

2 Freund, E., *Z. exp. Path. Ther.*, 1907, **4**, 1; Freund, E., and Popper, H., *Biochem. Z.*, 1909, **15**, 272.

3 Fine, J., and Seligman, A. S., *J. Clin. Invest.*, 1943, **22**, 265 and 285.

* We are indebted to Dr. Frey for the yeast strain and the personal communication of the details of the growing technic.

4 Schultz, A. S., Atkin, L., and Frey, C. N., *J. Biol. Chem.*, 1940, **135**, 267; Frey, C. N., private communication.

5 Meisenheimer, J., *Z. physiol. Chem.*, 1910, **104**, 229; 1921, **114**, 205.

6 Block, R. J., and Bolling, D., *Arch. Biochem.*, 1943, **3**, 217; Carter, H. E., and Phillips, G. E., *Fed. Proc.*, 1944, **3**, 123.

7 Wasteneys, H., and Borsook, H., *J. Biol. Chem.*, 1924-5, **62**, 1.

TABLE 1.
Distribution of N^{15} After Peptone Injection.

Mouse:	No. 1, 22 g		No. 8, 21 g		No. 20, 23 g		No. 3, 20 g		No. 10, 24 g		No. 21, 24 g		No. 11, 23 g	
Killed after:	10 min		10 min		10 min		30 min		60 min		60 min		180 min	
Injected	1.0 ml		.15 ml		.25 ml		.8 ml		.22 ml		.25 ml		.25 ml	
Organs	A. % Excess N^{15}.													
Liver	.014		.018		.038		.025		.027		.057		.021	
Gastro-Intestinal Tract	.019		.031		.021		.035		.033		.109		.034	
Blood, Spleen and Heart	.009		.022		.014		.018		.018		.034		.018	
Lung	.025		.017		.016		.031		.017		.034		.080	
Kidney	.028		.086		.030		.055		.018		.062		.113	
Uro-genital Tract	.191		.056		.067		.121		.019		.101		.689	
Skin	.053		.012		.071		.090		.026		.047		.029	
Carcass	.024		.011		.057		.032		.012		.036		.018	
Tail	.013		.063		.068		.016		.086		.045		.062	
Excreta	*		*		*		*		.598		.527†		.016	
Total excess N^{15} found in atoms $\times 10^{-6}$	12.2		7.2		26.0		11.7		12.3		24.4		14.3	
	B. Distribution of the N and N^{15} in % of the Total N and N^{15} of the Animal.													
	N	N^{15}	N	N^{15}	N	N^{15}	N	N^{15}	N	N^{15}	N	N^{15}	N	N^{15}
Liver	6.0	3.1	5.1	5.5	5.2	3.8	8.3	6.0	6.2	6.0	4.4	4.8	5.0	3.4
Gastro-Intestinal Tract	9.1	6.0	10.0	17.2	8.6	3.5	9.1	9.4	8.8	11.5	8.2	17.6	8.3	8.7
Blood, Spleen and Heart	4.6	1.4	3.5	4.2	6.0	1.7	5.0	2.6	4.6	3.0	3.9	2.7	4.4	2.7
Lung	0.9	0.6	0.7	0.8	0.7	0.2	0.8	0.6	0.7	0.5	0.6	0.5	0.7	1.7
Kidney	1.3	1.2	1.4	0.7	1.6	0.9	1.6	2.5	1.5	6.0	1.4	1.7	1.5	5.8
Uro-genital Tract	1.0	6.6	1.1	3.8	2.8	3.6	1.5	9.2	2.6	1.8	0.9	1.2	2.6	1.4
Skin	22.0	38.3	21.0	16.3	17.0	23.2	19.3	22.2	23.0	21.8	25.2	23.2	27.8	20.3
Carcass	50.2	41.8	53.0	37.3	54.4	58.2	50.7	45.8	48.3	27.0	51.3	35.2	51.0	29.3
Tail	4.9	2.2	4.2	14.0	3.8	4.9	3.7	1.7	4.3	13.7	4.2	3.9	3.7	7.7
Excreta	—	—	—	—	—	—	—	—	—	8.7	—	9.2	—	18.9

* No excreta.
† Urine only

60, and 180 minutes after injection. The bodies were immediately divided into the following fractions: 1, liver; 2, gastro-intestinal tract; 3, blood, spleen, and heart; 4, lungs; 5, kidneys; 6, urogenital tract (without kidney); 7, excreta; 8, skin; 9, carcass; 10, tail. Each fraction was digested with sulphuric acid and the total nitrogen determined. The N^{15} of each fraction was determined according to Rittenberg, Schoenheimer, *et al.*[8] The experimental error of the method is $\pm 0.003\%$ N^{15}. In these experiments all values up to 0.008% excess N^{15} were considered as not significant and values 0.09% to 0.10% as traces. The total N^{15} recovered was within 10% of the amount injected.

Table I-A gives the values for N^{15} concentration of each fraction at 10, 30, 60, and 180 minutes after the injection. Table I-B presents the distribution of nitrogen and N^{15} for each fraction in per cent of total recovered nitrogen and N^{15}.

Within 10 minutes after injection, N^{15} is rapidly removed from the blood stream and deposited primarily in skin and muscles (54-81%). Blood, spleen, and heart accounted for 2.6-3.0% of the injected N^{15}. The highest concentration of N^{15} was present in the urogenital fraction, although the total amount of N^{15} was low.

During the course of the experiments, the concentration and amount of N^{15} decreased in the carcass and gradually increased in kidneys, urogenital tract, and excreta, 8.7%-9.2% of N^{15} being excreted after one hour and 18.9% after 3 hours. The amount of N^{15} in blood, spleen, and heart remained low (2.6-3.9%), throughout the experiment. The gastrointestinal tract showed an increase of N^{15} after 30 minutes and one hour, and a decrease after 3 hours.

To establish the form in which N^{15} was excreted in the urine (see Table I, mouse No. 21), 2 groups of 3 fasting male mice (weight 20-25 g) received intravenously 1 ml of a 2% yeast peptone solution. Two hours after the injection, the urine of each group together with the washings was collected and brought to 1 ml. To remove the coloring matter 0.1 ml of a 10% lead acetate solution was added, the mixture heated 5 minutes at 100° and centrifuged.[9] Excess lead was removed from the supernatant fluid by careful treatment with aqueous sodium hydroxide. The solution gave a negative biuret reaction and no precipitate with tannic acid at pH 5 and 0°. Normal human urine containing 0.50% to .025% added peptone treated in the same way gave a positive biuret reaction.

Discussion. The relatively high concentration of N^{15} in skin and carcass as well as the presence of between 50 and 80% of the total N^{15} in these organs seems to indicate a depot function for these organs in nitrogen metabolism. This is indicated at least for proteoses and peptones, since no similar distribution for amino acids was found by Schoenheimer *et al.*[1] The short period (10 minutes) of the first experiment does not seem to be sufficient time to allow for much extensive alteration of the injected materials and it may be assumed that they were absorbed or deposited as such. Similar high values at after one and 3 hours for skin and carcass would corroborate this conclusion. Nevertheless transamination or breakdown of the injected material and resynthesis of the amino acids into body protein may have occurred. It is interesting to note that the N^{15} of the carcass decreases and remains fairly constant for the skin. This is more notable if the distribution of the total nitrogen and N^{15} are compared for each fraction. The N^{15} concentration in the skin is always higher than in the carcass. This would be consistent with the picture of the carcass as an active storage organ and the skin with a less active function.

There is also evidence for absorption of injected N^{15} into the gastrointestinal tract throughout the experiment. Comparison of the average amount of total nitrogen and N^{15} for each fraction after different time periods, indicates an increase in the N^{15} concentration in the liver and especially in the gastrointestinal tract during the first 60 minutes. After 3 hours both fractions especially the liver show a decrease in the concentration.

Summary. 1. Yeast peptone containing

[8] Rittenberg, D., Keston, A. S., Rosebury, F., and Schoenheimer, R., *J. Biol. Chem.*, 1939, **127**, 291.

[9] Freund, O., *Centr. inn. Med.*, 1901, **22**, 27.

excess N^{15} was injected intravenously into mice. 2. The N^{15} was rapidly removed from the blood stream and after 10 minutes was found principally in the skin and carcass. The remainder of the N^{15} was accounted for in the gastrointestinal tract, liver, and urogenital tract. 3. Thirty to 180 minutes after injection the predominant portion of the N^{15} still appeared in the skin, carcass, gastrointestinal tract and with progressing time in the excreta. 4. On the average the N^{15} in the carcass showed a decrease after one hour, while the gastrointestinal tract showed an increase during the first hour and a decrease after 3 hours.

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Rate of Flow and Cell Count of Rat Thoracic Duct Lymph.*

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In the course of studies on the action of adrenocorticotrophic hormone on the leucocyte count in the rat,[1] it was thought advisable to study directly the effect of hormone administration on the lymphocyte cell content of thoracic duct lymph in this animal form. Review of the literature did not, however, reveal any description of the technic of cannulation and collection of lymph in the rat. The present report describes a method of cannulation of the thoracic duct in the rat, and observations on the white cell count, and rate of flow of lymph in the normal, adult female rat.

Technic. Rats of the Long-Evans strain, of weight 200-250 g, unfasted, were employed. Preliminary studies indicated the difficulty of accurate identification of the lymphatic trunks in the neck so that it was necessary to identify the vessels by intraperitoneal injection of ½ cc of 1% trypan blue, half an hour before cannulation was attempted. This amount of dye was sufficient to stain all the main lymphatic trunks and to demonstrate the site of entrance of the lymph stream into the junction of internal jugular and subclavian veins with the left superior vena cava (in the rat). When familiarity with the lymphatic vessel arrangement was attained, further use of the dye was obviated.

The animals were anesthetized by intraperitoneal injection of 50-60 mg/kg B.W. of sodium pentobarbital in 1% solution. Further amounts were injected as necessary to maintain the anesthetic state over long periods of time.

Two types of cannulæ were employed. Capillary tubes were used for drop-wise collection of the lymph. In other cases, 3 to 5 mm glass tubing was drawn out into a capillary tube at one end for the collection of larger samples of lymph. The capillary tubings were drawn to the size, externally, of No. 25 G-No. 27 G hypodermic needles, which were employed to make openings in the lymph vessels previous to insertion of the cannula. All manipulations and cannulation were carried out under the binocular dissecting microscope. Cannulæ were heparinized to prevent clotting. A midline incision was carried through skin and subcutaneous tissue of the neck, through the deep fascia, separating the submandibular salivary glands, dissecting to the ventral aspect of the sternohyoid muscles. Using a small pair of bone forceps, the manubrium sterni was split in the midline as far as the sternal angle. The two halves of the manubrium were retracted laterally by means of clamps and held apart exposing the length of

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1 Reinhardt, W. O., Aron, H., and Li, C. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 19.