A STUDY OF AMINO NITROGEN AND GLUCOSE IN LYMPH AND BLOOD BEFORE AND AFTER THE INJECTION OF NUTRIENT SOLUTIONS IN THE INTESTINE.

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INTRODUCTION.

The question of the absorption of protein by way of the lymph has not been considered since the true significance of protein digestion has been known. The earlier investigators were limited to the determination of total nitrogen as a means of following the products of protein digestion through the body. Newer methods make it possible and desirable to follow resorption of the products of protein digestion by the determination of amino nitrogen in the tissues and fluids of the body.

Asher and Barbera (1) fed egg albumin and by determining the apparent increase in total nitrogen in the lymph, thought they showed that a small amount of protein is absorbed through the lacteals. Mendel (2) repeated this experiment of Asher and Barbera, giving the animal Witte's peptone instead of egg albumin. His results showed a slight increase of total nitrogen in the lymph, but a smaller increase than that obtained by the former. He was inclined to believe that the lymph does not take up any protein from the intestines, as his slight increase in nitrogen seemed to be within the limits of experimental error. Howell (3) has demonstrated the presence of amino-acids in the lymph, but so far as we are aware, no quantitative determination of amino nitrogen in the lymph has been reported.

The presence of glucose in the lymph has been known for a long time, and as early as 1862 Gorup-Besanez (4) pointed out the fact that the percentage of sugar in the lymph is greater than in the blood. Ginsberg (5) thought the sugar in the blood is to be found in the plasma alone. He argued from this assumption that the lymph glucose did not show a real concentration over that of the blood but only an apparent one, since the removal of the red corpuscles, which make up from 30 to 40 per cent of the volume of the blood, would leave all of the sugar in the plasma, and, as he believed, in the lymph. However, Michaelis and Rona (6, 7) and von Hess and McGuigan (8) have shown that the amount of glucose in the red corpuscles is the same as in the plasma. Some other explanation must be sought for the relatively large amount of glucose in the lymph.

Von Mering (9) thought that the amount of glucose in the lymph is unaffected by a meal rich in sugar, but Ginsberg, working in Heidenhain's laboratory, showed that the amount of glucose in the lymph increases after sugar is injected into the intestine. He used dogs and rabbits and found the increase in both species of animals. Still he does not seem to have believed that sugar is resorbed directly by the lymph, but that it is first taken up by the blood and then secreted into the lymph spaces.

EXPERIMENTAL.

It has seemed worth while to make amino nitrogen determinations on blood and lymph collected before and after the injection, into the small intestine, of protein and amino-acid solutions and then compare the results with those obtained when sugar was injected and the glucose content of the blood and lymph determined. Dogs which had fasted about 18 hours were used in these experiments. They were anesthetized with ether, no morphine salts having been used.

Surgical Technique.—The ordinary thoracic fistula was used occasionally for the collection of lymph, but usually the collection was made through the left external jugular vein. This vein was tied off just below the entrance of the subclavian and also some distance above (at about the level of the upper edge of the thyroid gland); then all the veins (including the subclavian) flowing into the jugular between these ligatures were tied and cut. After this was done, an incision was made in the jugular, and in a few moments perfectly bloodless lymph flowed from a cannula inserted into the vein.

The lymph, thus obtained, came not only from the thoracic duct but also from the lymph vessels arising in the head and neck; however, our results did not show any difference between the samples of lymph collected in the different ways.

The operation, in preparing this type of lymphatic fistula, has the advantage of being more quickly performed by an experienced surgeon, and is one which a physiologist can do with little difficulty after a few attempts. The thoracic duct seems, at times, to end in a sort of "delta," and under these conditions, the collection through the jugular vein is much more complete than it would be by the usual method.

Chemical Methods.—In preparing the blood and lymph for the amino nitrogen determination, the method of Van Slyke and Meyer (10) was followed. Usually 15 cc. of the blood or lymph were run into 85 cc. of 95 per cent alcohol and then 40 cc. of the alcohol added to make the volume up to 140 cc. The amino nitrogen was determined by the well known micro method of Van Slyke (11). The method of Lewis and Benedict (12) was followed in the glucose determinations.

Experimental Results.—The results of four experiments are given in the following tables. In two of these experiments, solutions of Fairchild Brothers and Foster's "peptone" were used; in one of the others, milk was used; and in the fourth, a solution of amino-acids was injected. These injections were all made into the intestine at about the level of the lower end of the pancreas. It is to be noted that the quantity of protein used was relatively large in each case, probably somewhat in excess of the amount present in the intestine at any time under normal conditions.

Experiment 1. March 21, 1917.

Operation complete, 10.15 a.m.

Sample No.	Material.	Time of collection.	Amino N per 100 cc.
			mg.
1	Blood.	10.30	3.6
2	Lymph.	10.15–10.30	1.9
Injected 500 cc. of 10 per cent "peptone" solution at 10.40.			
3	Lymph.	10.30–10.45	4.0
4	"	10.45–11.00	6.5
5	Blood.	11.00	10.2
6	Lymph.	11.00–11.14	13.8
7	"	11.14–11.28	19.0
8	Blood.	11.28*	4.7

* Animal dead.

Experiment 2. April 12, 1917.

Operation complete, 10.20 a.m.

Sample No.	Material.	Time of collection.	Amino N per 100 cc.
			mg.
1	Lymph.	11.00–11.18	Lost.
2	Blood.	11.13	3.4
3	Lymph.	11.08–11.20	3.1
Injected 500 cc. of milk at 11.29.			
4	Lymph.	11.20–11.31	2.9
5	"	11.31–11.44	3.6
6	"	11.44–11.51	3.2 (?)
7	"	11.51–11.58	Lost.
8	"	11.58–12.06	4.9
9	Blood.	12.09	4.4
10	Lymph.	12.06–12.13	6.1
11	"	12.13–12.22	7.2
12	"	12.22–12.35	8.3
13	"	12.35	6.6
14	"		4.5
15	"	12.55–1.02	4.4
16	Blood.	1.02	3.2

Experiment 3. April 23, 1917.

Operation complete, 11.15 a.m.

Sample No.	Material.	Time of collection.	Amino N per 100 cc.
			mg.
1	Lymph.	11.15–11.55	3.7
2	Blood.	11.55	5.3
Injected solution of amino-acids containing 4.2 gm. amino nitrogen solution at 12.00.			
3	Lymph.	11.55–12.20	6.0
4	"	12.20–12.40	9.2
5	Blood.	12.45	6.5
6	Lymph.	12.40–1.00	9.7
7	Blood.	1.20	6.5
8	"	1.20	8.6 (?)

Experiment 4. May 16, 1917.

Operation complete, 11.15 a.m.

Sample No.	Material.	Time of collection.	Amino N per 100 cc.
			mg.
1	Lymph.	11.15–11.40	4.7
2	Blood.	11.45	6.3
Injected 500 cc. of 5 per cent "peptone" solution at 12.30.			
3	Lymph.	12.30–1.05	8.2
4	"	1.07–1.28	6.0 (?)
5	Blood.	1.10	6.4
6	Lymph.		8.7
7	Blood.	1.42	4.9
8	Lymph.		6.4
9	Blood.		6.8
10	Lymph.		7.4

It will be observed from the tables given above that, in the fasting dog, the amount of amino nitrogen in the lymph was less than in the blood. Sometimes the difference was quite marked, while at other times, it was insignificant. This is the reverse of the relation of the amount of glucose in the blood and lymph. After the protein or amino-acid solution is placed in the intestine, the amino nitrogen content of the blood and lymph is increased. Although this increase was not always quite regular, the increase in the blood was only once or twice more than 50 per cent, while in the lymph, the increase was usually more than 100 per cent and in many cases much greater. In Experiment 1, the amino nitrogen in the lymph rose to a trifle over 19 mg. per 100 cc., which is practically ten times what it was in the control period. It is to be pointed out that, after the injection of protein or amino-acid solution into the intestine, not only the increase in, but also, the absolute amount of amino nitrogen in the lymph, becomes greater than in the blood.

It is to be noted that the blood was collected from the general circulation, sometimes from the jugular vein, and at other times from the femoral artery. Since the liver is believed to take up a portion of the amino acids from the portal blood, possibly, if the blood had been collected from the portal or one of the mesenteric veins, there would have been little or no difference in the amino nitrogen content of the blood and lymph.

These experiments would seem to show that the amino-acids in the intestine may be taken up by both blood and lymph. They also suggest that the amount taken up by each of these fluids is dependent upon the volume of the two fluids which are flowing through the walls, and especially, the villi of the intestine during the period of resorption. This would mean, of course, that only a relatively small amount of protein nitrogen is taken up by the lymph, since the amount flowing from the intestine is certainly small as compared with the flow of blood.

We have already mentioned the work of Ginsberg in which he showed that the amount of glucose in the lymph increases after the injection of sugar into the intestine. The following tables from three of our experiments show that we have been able to confirm these results. These experiments were carried out in exactly the same way as those in which we were studying the resorption of amino-acids, except that glucose solutions were introduced into the intestine and 2 cc. samples of blood and lymph were collected for glucose determinations.

Experiment 5. May 29, 1917.

Operation complete, 11.45 a.m.

Sample No.	Material.	Time of collection.	Per cent of glucose.
1	Lymph.	12.25	0.225
2	"	12.30	0.225
3	Blood.	12.35	0.145
4	"	12.35	0.145
Injected 50 gm. glucose dissolved in 250 cc. of water at 1.05.			
5	Lymph.	1.15	0.250
6	Blood.	1.15	0.136
7	Lymph.	1.30	0.321
8	Blood.	1.35	0.176
9	Lymph.	2.00	0.321
10	Blood.	2.05	0.180
11	Lymph.	2.30	0.310

Experiment 6. June 15, 1917.

Operation complete, 10.45 a.m.

Sample No.	Material.	Time of collection.	Per cent of glucose.
1	Lymph.	10.50	0.300
2	Blood.	10.55	0.176
Injected 50 gm. glucose dissolved in 250 cc. of water at 11.00.			
3	Lymph.	11.20	0.306
4	Blood.	11.20	0.228
5	Lymph.	11.45	0.360
6	Blood.	11.55	0.247
7	Lymph.	12.10	0.434
8	Blood.	12.10	0.271

Experiment 7. August 10, 1917.*

Operation complete, 11.10 a.m.

Sample No.	Material.	Time of collection.	Per cent of glucose.
1	Lymph.	11.15	0.173
2	Blood.	11.19	0.125
Injected 50 gm. glucose dissolved in 250 cc. of water at 11.20.			
3	Lymph.	11.35	0.164
4	Blood.	12.20	0.281
5	Lymph.	12.35	0.300
6	Blood.	12.50	0.300
7	Lymph.	1.03	0.281

* In this experiment, blood was collected from the mesenteric veins.

Special attention should be called to Experiment 7. It was carried out just as the other glucose experiments except that the blood samples *were collected from the mesenteric veins.* This experiment indicates, if it does not clearly show, that the glucose content of the thoracic lymph and the portal blood is the same. This does not account for the amount of glucose in the lymph of the fasting dog. None of these experiments throw any light on that fact.

It would seem, on purely physical grounds, that the concentration of glucose and of amino-acids in the lymph and blood of the villi of the intestine would be identical, as the processes of diffusion and osmosis would operate to eliminate any inequalities, unless a semipermeable membrane is present to prevent the passage of these substances in one direction or the other. As it is generally agreed that these substances pass into the blood through the walls of the capillaries, there does not appear to be any physical basis for believing that they would pass into the capillaries to such an extent that the blood would be richer in these compounds than the fluid which is found in the intracellular spaces surrounding the blood vessels. Heidenhain (13) thought the structure of the villi of the intestine practically prevents the entrance of sugar and other crystalloids into the lymph, as he, as well as Schäfer (14) and others, have shown that the blood vessels lie adjacent to the basement membrane of the epithelial cells which cover the villi, while the lacteal is centrally located. He did not take account of the fact that the intracellular spaces are really continuous with the lymphatic system so that, in reality, the blood system can be regarded as no nearer to the resorbing surface than the lymphatics.

Therefore, it is suggested that the practically complete resorption of sugar and amino-acids by the blood is to be accounted for by the almost infinitely large volume of blood flow as compared with that of the lymph.

SUMMARY.

1. Less amino nitrogen is found in the thoracic lymph than in the blood of a fasting dog.

2. After the injection of milk, "peptone," or amino-acid solutions into the intestine, the amino nitrogen in both the systemic blood and lymph increases, but the amount in the lymph is greater than in the blood.

3. Ginsberg's findings, that the introduction of sugar solutions into the intestine increases the amount of glucose in the lymph, are confirmed. The old observation, that the amount of glucose in the lymph is greater than in the blood, has also been confirmed.

4. The amount of sugar in the blood of the mesenteric veins and the lymph after the introduction of sugar into the intestine, seems to be practically the same.

5. It is suggested that the practically complete absorption of protein and carbohydrate by the blood is not due to a selective resorption, but to the almost infinitely large volume of blood, as compared to the volume of lymph, which flows through the walls of the intestine.

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