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Metabolism of Intraperitoneally Injected Serum Protein.*

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Two groups of 10 albino rats with identical average body weights were selected. For 2 days they were given no food. On the morning and evening of each day 12 cc of 6.4% dextrose in 0.9% sodium chloride was injected into the peritoneal cavities of the controls while to the experimental group an injection of 12 cc of freshly separated serum obtained from normal rats was given. Both groups were anesthetized 48 hours after the first injection and 18 hours after the last. All the blood that could be obtained from the cut abdominal aorta while the heart was still beating was centrifuged at high speed for half an hour and the serum separated from the clot. The quantity thus drawn represents about 75% of the total blood volume. The liver, kidneys and heart were then removed, weighed and prepared for the protein determinations. The contents of the alimentary tract were removed after boiling in 0.5 M acetate buffer at pH 5. and the protein content of the tract determined with the carcass.[1] In the experimental group the abdominal cavities contained on an average nearly 5 cc of a fluid that had a somewhat higher protein concentration than the serum that had been injected. This fluid was collected and its protein is not included in the total protein given in Table I.

TABLE I.
Effect of Intraperitoneal Injection of Serum on Total Organ Protein.

	Control—Dextrose Injection		Experimental—Serum Injection	
	Wt of organ	Protein of organ	Wt of organ	Protein of organ
Liver	6.451	1.235	7.356	1.522
Kidneys	1.279	0.199	1.492	0.235
Heart	0.751	0.121	0.765	0.132
Serum	3.490	0.212	4.495	0.427
Clot	3.315	0.861	3.560	0.992
Carcass and Alimentary Tract	196.081	32.015	205.717	34.430
Total	211.367	34.643	223.385	37.738

* This work was aided by a grant from the Rockefeller Foundation.

1 Addis, T., Poo, L. J., Lew, A., and Yuen, D. W., *J. Biol. Chem.*, 1936, **113**, 497.

Since the original body weights of the two groups were identical the quantities found after 48 hours may be directly compared. It is evident that in all the organs examined serum injection is associated with a higher protein content than is found after dextrose injection. But the only important question is whether this greater protein content is a part of the rat's protein or is only the serum protein that we had injected. Now it will be noted that the serum content of the experimental group is double the serum content of the control group. This is due partly to an increase in the quantity of serum in the serum-injected group but mainly to an increase in protein concentration from 6.1% in the controls to 9.5% in the experimentals. It is reasonable, therefore, to consider that this singular hyperproteinemia is a passive phenomenon and represents the circulation of the injected serum protein that has been absorbed but not yet assimilated. However, the quantity of this presumably unassimilated protein is only 0.215 g while the total increase in protein distributed through all the exsanguinated organs and tissues of the serum-injected group is 3.095 g. In addition it must be remembered that the total amount of protein injected in the form of serum (after subtraction of what was found unabsorbed in the abdominal cavity and the small amount excreted in the urine), was only 2.417 g. The differences we observe cannot well be explained by a simple retention of unaltered serum protein. They are explicable on the assumption that the injected protein was metabolized. They thus extend the original demonstration of Holman, Mahoney and Whipple[2] that, in the dog, intravenously injected protein is utilized. On the other hand, the hypothesis advanced by Howland and Hawkins[3] that the injected protein is not broken down into amino-acids but is incorporated into the body proteins in the form of large aggregations of amino-acids has been rendered untenable by the work of Bergmann,[4] who has shown that each protein has its own individual periodicity in the arrangement of its amino-acids and by the work of Schoenheimer, *et al.*,[5] who have found that even indispensable amino-acids undergo intramolecular rearrangement before they are finally incorporated within the pattern of the amino-acid chains that constitute the proteins of the body. Howland and Hawkins were led to their view of the incomplete disintegration of the injected protein because

[2] Holman, R. L., Mahoney, E. B., and Whipple, G. H., *J. Exp. Med.*, 1934, **59**, 269.

[3] Howland, J. W., and Hawkins, W. B., *J. Biol. Chem.*, 1938, **123**, 99.

[4] Bergmann, M., *Chem. Rev.*, 1938, **22**, 423.

[5] Schoenheimer, R., Ratner, S., and Rittenberg, D., *J. Biol. Chem.*, 1939, **127**, 333.

in phloridzinized dogs they did not find that protein injection was followed by the increased N_2 and sugar excretion that followed the taking of similar quantities of protein by mouth. But since Drury[6] has demonstrated the utilization of dextrose in phloridzinized dogs it is possible to suppose that the difference may be an indication of slow digestion of protein to amino-acids, not of no digestion at all. The preliminary hypothesis on which our future experiments will be based is that the injected protein is taken up by the reticulo-endothelial cells of the body and subjected to intra-cellular digestion to amino-acids.

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Utilization of Amino-Acids by *Clostridium botulinum.**

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Our knowledge of the metabolism of *Clostridium botulinum* is at present scanty. Wagner, Meyer and Dozier[1] analyzed the products of the action of *Cl. botulinum* on complex media but obtained little information as to the reactions by which the products had been formed. Knight,[2] summarizing the growth-requirements of *Cl. botulinum,* reports that the simplest medium which will support growth contains the amino-acids proline, glycine, leucine, alanine, lysine, and cystine, together probably with traces of tryptophan and of the "sporogenes growth factor." He suggests that *Cl. botulinum* may obtain its energy through the "Stickland reaction," a coupled oxido-reduction between pairs of amino-acids. Glycine and proline act as hydrogen acceptors while alanine and leucine act as hydrogen donators in the case of *Cl. sporogenes* (Stickland[3]). Therefore these amino-acids were tested to determine whether or not they are attacked directly as is true for *Cl. tetanomorphum* with various

6 Drury, D. R., Bergmann, D. C., and Greeley, P. O., *Am. J. Physiol.*, 1939, **117**, 323.

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1 Wagner, E., Meyer, K. F., and Dozier, C. C., *J. Bact.*, 1925, **10**, 321.

2 Knight, B. C. J. G., *Bacterial Nutrition*, 1936, 117-120, His Majesty's Stationery Office, London.

3 Stickland, L. H., *Biochem. J.*, 1934, **28**, 1746.