strated on animals maintained on adequate levels of protein[8] and that of casein and especially of the amino acids methionine and cystine on rats receiving adequate amounts of vitamins.[9]

[8] Antopol, W., and Unna, K., *Cancer Research*, 1942, **2**, 694.

[9] György, P., Poling, E. C., and Goldblatt, H., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 41.

Summary. Rats maintained on a low protein diet were unable to retain normal amounts of riboflavin in the liver. Methionine tended to counteract the decrease in the riboflavin concentration of the liver. The livers of rats maintained on a low protein diet for a period of 3 months failed to inactivate estradiol. Supplementation with methionine maintained the ability to inactivate estradiol.

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Acetylcholine Content of the Myenteric Plexus and Resistance to Anoxia.

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It has been pointed out in an earlier paper[1] that the order of increasing resistance of the parts of the mammalian nervous system to anoxia and hypoglycemia is essentially the same as the order of parts arranged according to increasing amounts of acetylcholine (ACh) per unit weight. Those parts which are least resistant (cerebellum and cortex) are low in ACh and those which are among the more resistant (spinal nerves and autonomic ganglia) are high in ACh.

Cannon and Burket[2] have shown the cells of the myenteric plexus to be highly resistant to oxygen lack, enduring 3 hours of complete anemia. Van Liere[3] has found the alimentary canal to be affected significantly only by a strong degree of anoxia. It is of interest, therefore, to know the normal level of ACh in the myenteric plexus. Dikshit[4] has shown that the rate of formation of ACh by pieces of intestine *in vitro* is as high as that of equivalent amounts of nervous tissue but, so far as we are aware, no one has attempted to estimate the ACh-content of the enteric plexuses. This we have done for Auerbach's plexus in the rabbit and guinea pig and it will be seen that, in these species, the ACh-content of this plexus is extraordinarily high.

Methods. Tissue for assay was obtained by stripping off the longitudinal muscle and serosa of the entire small intestine of adult guinea pigs and rabbits. Most of Auerbach's plexus adheres to the longitudinal muscle. Assays were also made on pieces of whole intestine. The tissues were rinsed, carefully blotted to remove excess moisture, and weighed. They were ground, with a small amount of a phosphate medium plus eserine 1:5,000, acidified to pH 3-4 with normal HCl, and allowed to stand for one hour or more. They were then neutralized with normal NaOH and the volume was adjusted to give 100 mg tissue/cc fluid. After centrifuging, the supernatant fluid was further diluted for assay. This method gives the "total" ACh. Assays were made using the ventricle preparation ("heart") from Venus mercenaria, or the frog's rectus abdominis muscle. An estimate of the relative proportion of nervous to non-nervous tissue in the removed outer layers was obtained by projecting a stained preparation of the myenteric plexus of the rabbit and making a tracing. Cutting out and weighing the parts gave an estimate of the area of the plexus and the thickness was obtained from cross sections. This method indicated that

[1] Welsh, J. H., and Hyde, J. E., *J. Neurophysiol.*, 1944, **7**, 41.

[2] Cannon, W. B., and Burket, I. R., *Am. J. Physiol.*, 1913, **32**, 347.

[3] Van Liere, E. J., *Anoxia*, Univ. Chicago Press, 1942.

[4] Dikshit, B. B., *Quart. J. Exp. Physiol.*, 1938, **28**, 243.

TABLE I.
Acetylcholine Content of Small Intestine and Outer Layers (Myenteric Plexus, Longitudinal Muscle and Serosa) of Rabbit and Guinea Pig.
(Figures in parentheses represent values obtained from assays using frog's rectus, those not in parentheses from assays using the Venus' heart.)

	Whole intestine, γ ACh/g		Outer layers, γ ACh/g	
Rabbit No. 1	—		16	
" " 2	3.3		10	(15)
" " 3	4.0	(4.0)		(10)
Guinea pig 1	—		20	
" " 2	6.5		16	
" " 3		(8)		(20)
" " 4		(10)		(20)
" " 5	4.0		—	
" " 6	7.5		—	
" " 7	8.6		—	

the plexus constitutes roughly 1/40 of the total mass of nerve, muscle, and serous tissues.

Results. The results are given in the accompanying table. The values for total ACh in the small intestine of the rabbit (3 3 and 4 γ/g) correspond closely with those obtained by Chang and Gaddum[5] (2.8 and 4 γ AChCl/g). The extracts of the longitudinal muscle and serosa with the attached plexus gave values for the rabbit ranging from 10 to 16 γ/g. These latter values must be multiplied by 40 to obtain an estimate of the ACh per gram of Auerbach's plexus. This is assuming that all of the ACh is in the plexus, for which there is good evidence.[4]

Intestinal tissue of the guinea pig yielded values ranging from 4 to 10 γ ACh/g (avg 7.4 γ); while the removed outer layers, with the attached plexus, gave values of 16 and 20 γ ACh/g. Dikshit[4] found that guinea pig intestine synthesized ACh at a rate twice that of rabbit intestine. This difference in rate corresponds with the difference in ACh content of the intestines of the two species (rabbit, avg 3.8 γ/g; guinea pig, avg. 7.4 γ/g). No attempt was made to estimate the relative weight or mass of the myenteric plexuses in the guinea pig and rabbit, hence it is not known whether the higher values for the former are due to a proportionately greater amount of nerve tissue or a higher level of ACh in a given amount of tissue.

Discussion. To obtain an estimate of the ACh per gram of Auerbach's plexus of the rabbit one must multiply the values obtained for extracts of the outer layers by 40. This yields values ranging from 400 to 640 γ/g of plexus. Assuming the proportions of ganglionic to muscle and serous tissues to be the same in guinea pig and rabbit, corresponding values for Auerbach's plexus in the guinea pig run as high as 800 γ/g. The highest values obtained previously for parts of the mammalian nervous system are those for autonomic ganglia. MacIntosh[6] found values ranging from 12-44 γ/g in autonomic ganglia of the cat. Feldberg[7] obtained values for the superior cervical ganglia of the cat ranging from 23.5 to 36.8 γ/g.

The gray matter of the cerebrum of the cat has an ACh content of 1.2-4.5 γ/g[6] and fails to recover after periods of complete arrest of body circulation of 3-7 minutes.[8] Superior cervical ganglia of the cat have an ACh content about 10 times that of cortical gray matter and survive oxygen lack for 60-70 minutes.[9] Calculated from the rate of ACh synthesis, the myenteric plexus of the cat appears to have about the same ACh content as the rabbit[4] (roughly 10 times that of cervical ganglia) and survives complete anoxia for 3 hours.[2] Thus further evidence is provided indicating a correlation between ACh content and resistance to anoxia in parts of the nervous system.

Summary. It is shown that the estimated ACh-content of the myenteric plexus of the rabbit and guinea pig (400-800 γ/g) is higher than that of any other mammalian nervous tissue. Since the cells of this plexus are known to be the most resistant of all nerve cells to anoxia, further evidence is provided indicating a relationship between resistance to oxygen-lack and ACh-content of parts of the nervous system.

[5] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[6] MacIntosh, F. C., *J. Physiol.*, 1941, **99**, 436.

[7] Feldberg, W., *J. Physiol.*, 1943, **101**, 432.

[8] Weinberger, L. M., Gibbon, M. H., and Gibbon, J. H., Jr., *Arch. Neurol. and Psychiat.*, 1940, **43**, 961.

[9] Bargeton, D., *Am. J. Physiol.*, 1938, **121**, 261.