Nielsen and Black did not incorporate p-aminobenzoic acid in their rations does not offer a convincing reason for the discrepancy. It seems much more likely that the difference is due to the nature of the dietary carbohydrate. We have used dextrose while Nielsen and Black used sucrose. This aspect of the problem is now being further investigated. Another possible explanation at the moment seems to be one of strain difference. Our evidence[4,5] very strongly supports the view that there is a clear strain difference in the nutritional requirements of the C_{57} and the A strain. The mice used by Nielsen and Black may have possessed such a high requirement for biotin and folic acid that they showed deficiency symptoms during a very early period of life.

The difference in the time required for A strain females to develop mammary tumors on the stock and the synthetic diets is highly suggestive and is being investigated more thoroughly.

The much greater incidence of symptoms and deaths of A strain mice on synthetic diets suggests again their greater nutritional requirements. We have shown in this laboratory[4,5] that this strain requires more riboflavin and pantothenic acid for growth than does the C_{57} strain. The findings reported here suggest also a greater need for biotin or folic acid or both in order to maintain the animal in good health during later life. The absence of possible unknown nutritional factors cannot be altogether overlooked. We have found[6] that mice of the C_{57} strain on synthetic diets had greater cecal contents and a greater bacterial population than did mice of the A strain. This strain difference was not observed in animals on stock ration.

Summary. Two strains of highly inbred mice, one tumor-susceptible and the other tumor-resistant, were maintained for a period of about one year on a stock diet or on a synthetic ration adequate for good growth performance. The tumor-susceptible A strain on synthetic diet showed numerous deficiency symptoms not seen on stock rations and not shown to any great extent by C_{57} mice on either stock or synthetic diets.

[4] Fenton, P. F., and Cowgill, G. R., *J. Nutrition*, 1947, **34**, 273.

[5] Fenton, P. F., and Cowgill, G. R., *Fed. Proc.*, 1947, **6**, 407.

[6] Gall, L. S., Fenton, P. F., and Cowgill, G. R., in press.

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Antagonism of Sulfadiazine Inhibition of Psittacosis Virus by p-Aminobenzoic and Pteroylglutamic Acids.*

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Studies on the chemotherapeutic action of the sulfonamides on certain bacteria[1] have shown that p-aminobenzoic acid (PABA) and pteroylglutamic acid (PGA) antagonize the inhibition of growth of these organisms by sulfonamide drugs. The demonstration of sulfadiazine (SD) inhibition of the growth of certain strains of psittacosis virus[2] suggests the likelihood that PABA and PGA might be active antagonists for this inhibitory effect of the sulfonamides.

A yolk sac passage strain of psittacosis

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[1] Lampen, J. O., and Jones, M. J., *J. Biol. Chem.*, 1947, **170**, 133.

[2] Early, R. L., and Morgan, H. R., *J. Immunol.*, 1946, **53**, 151.

virus (6BC) was employed using the same experimental techniques previously described[2] for the determination of the effect of SD on the growth of the virus. PABA and PGA were prepared as sterile solutions of their sodium salts in distilled water. These solutions were mixed with the proper amounts of a sterile solution of sodium sulfadiazine just before injection in 0.25 ml amounts into the yolk sac of 7-day-old embryonated eggs. This was followed in ½ hour by the injection of a yolk sac suspension of psittacosis virus diluted to contain 10,000 LD_{50} in 0.25 ml. The doses of the various compounds are recorded in weights of PABA, PGA and SD.

Preliminary experiments have indicated that the minimal amounts of the two compounds required to antagonize the inhibitory effect of 2.5 mg SD on the growth of the virus was 0.005 mg PABA and 0.05 mg PGA. Following the establishment of these relationships, a series of experiments was carried out to determine the amounts of the 2 inhibitors required to antagonize the action of larger amounts of SD. Representative results are presented in Table I, using amounts of PABA and PGA twice and ten times the minimal doses noted above.

There is a direct relationship between the amount of SD used and the amount of PABA required to antagonize its inhibitory action. This suggests a competitive inhibition of the action of SD. In contrast, a given dose of PGA is demonstrated to be actively antagonistic for increasingly larger doses of SD which suggests a noncompetitive antagonism. These data suggest that, as is the case with certain bacteria which synthesize PGA,[1] the primary action of SD on psittacosis virus is directed against the incorporation of PABA into PGA by the virus because of the direct relationship between the amount of PABA required to antagonize a given amount of SD. When PGA is supplied, even large doses of SD fail to inhibit the growth of the virus. These findings suggest that psittacosis virus (6BC strain) synthesizes PGA and that this vitamin is required for its growth. Studies are now underway to elucidate this possible metabolic activity of the virus.

TABLE I.
Antagonism of Sulfadiazine Inhibition of Psittacosis Virus by *p*-Aminobenzoic Acid and Pteroylglutamic Acid.

SD, mg	Inhibitor agent, mg		No. eggs	% survived 10 days*
0.5	PABA	.01	7	0
2		,,	8	0
5		,,	6	0
10		,,	19	47
25		,,	13	85
50		,,	6	100
0.5	PABA	.05	5	0
2		,,	8	0
5		,,	8	0
10		,,	18	0
25		,,	11	8
50		,,	4	100
0.5	PGA	.1	7	0
2		,,	8	0
5		,,	6	0
10		,,	16	0
25		,,	11	0
50		,,	8	0
0.5	PGA	.5	8	0
2		,,	7	0
5		,,	7	0
10		,,	20	0
25		,,	10	0
50		,,	7	0
0.5		—	16	82
5		—	24	96
—	PABA	5.0	8	0
—	PGA	10.0	10	0
—		—	16	0
		Drug Controls.†		
50		—	8	85
—	PABA	5	20	95
—	PGA	10	19	95

* 10,000 LD_{50} injected into each egg.
† Not infected.

Summary. The chemotherapeutic action of sulfadiazine on psittacosis virus (strain 6BC) is antagonized competitively by p-aminobenzoic acid and noncompetitively by pteroylglutamic acid. The implications of these findings with regard to the metabolic activities of this virus are discussed.