lationship has been demonstrated in the case of vitamin B_6 and pantothenic acid. Much more work is necessary before we can establish the true relationship between vitamin deficiencies and the respiratory quotient. But these preliminary experiments do indicate that studies along this line may be profitable and lead to a clearer understanding of the relation of vitamin deficiency to energy metabolism.

Summary. Riboflavin and pantothenic acid deficiencies in rats do not alter the BMR. A definite decrease in the BMR was observed in severe vitamin B_6 deficiency. All rats showing a riboflavin, vitamin B_6, and pantothenic acid deficiency had a higher RQ than normal rats. The possible explanation for this increased RQ is given.

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Production of a Diazotizable Substance by *Escherichia coli* during Sulfonamide Bacteriostasis.

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The classical paper of Woods,[1] describing the anti-sulfonamide action of p-aminobenzoic acid, crystallized the concept that sulfonamide bacteriostasis is the result of some interference with the normal metabolism of susceptible bacteria.[2] Woods[1] and Fildes[3] suggested that the p-aminophenyl grouping of the sulfonamides is sufficiently similar to that in the hypothesized metabolite, p-aminobenzoic acid, so that competitive inhibition occurs when the relative concentrations of PAB/Drug approach 1/5000. Our recent studies on the ionization of sulfonamides[4] seem to indicate that in terms of concentration of anions this ratio is 1/1 - 1/6.

Up to the present p-aminobenzoic acid has not been found essential for the normal growth of pathogenic bacteria nor has it been detected in such bacteria. During sulfonamide bacteriostasis, on the other hand, another diazotizable aromatic amine appears in the medium. The circumstances of its appearance are described in this preliminary note.

Synthetic medium* containing bacteriostatic concentrations of sulfonamide drugs[5] was placed in flasks or test tubes and inoculated with *Es. coli* (final dilution = 1 to 5 million per cc). Control samples taken at once were refrigerated and the remainder incubated at 37°C for 18 hr. Turbid cultures were centrifuged and diazo tests† were then done on the supernatant and the control samples. A typical result is as follows:

*Ammonium citrate	5	g
Magnesium sulfate	1.0	"
Sodium carbonate	3.0	"
Potassium dihydrogen phosphate	3.0	"
Sodium chloride	2.0	"
Iron ammonium citrate	.05	"
Nicotinic acid	.005	"
Glucose	.2	"
Acid hydrolysate of gelatin (Knight)	10	cc
Tap water to 1000 cc.		

[1] Woods, D. D., *Brit. J. Exp. Path.*, 1940, **21**, 74.

[2] (*a*) Mayer, R. L., *Bull. d. l'Acad. d. med.*, 1938, **117**, 727; (*b*) McIntosh, J., and Whitby, L., *Lancet*, 1939, **1**, 431; (*c*) Lockwood, J. S., *J. Immunol.*, 1938, **35**, 155; (*d*) Fox, C. L., Jr., *Am. J. Med. Sci.*, 1940, **199**, 487; (*e*) Mellon, R. R., Locke, A. P., and Shinn, L. E., *Am. J. Med. Sci.*, 1940, **199**, 749.

[3] Fildes, P., *Lancet*, 1940, **1**, 955.

[4] Fox, C. L., Jr., and Rose, H. M., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 142.

[5] Rose, H. M., and Fox, C. L., Jr., *Science*, 1942, **95**, 412.

† The method of Bratton and Marshall was used.[6] This test is not specific for sulfonamides; positive results are obtained with most primary aromatic amino compounds.

Initial control diazo test	0.848 mg% sulfadiazine
After incubation diazo test	1.94
Increment	1.09 mg% diazotizable substance

Apparently significant amounts of diazotizable substances are produced during bacteriostasis. Control experiments showed that none is formed in the absence of either drug or bacteria. It is difficult to conceive of a chemical basis for intensification of the diazo color by the action of the bacteria on the drug itself. Nothing more can be said at present as to the source of the newly formed aromatic amine.

Obviously the first thought as to the identity of the new substance was p-aminobenzoic acid for this compound when diazotized and coupled yields as much color as an equimolar amount of sulfonamide. To investigate this possibility the new substance was obtained in sterile medium in this manner:

Synthetic medium containing bacteriostatic concentrations of sulfonamide drugs[5] was placed in cellophane bags‡ (ca 100 cc). In one series the sacs were tied off and immersed in 1 L. of the same drug medium in 3 liter Erlenmeyer flasks; in other series the sacs were fastened to 10 cm lengths of 5 mm glass tubing which passed through the cotton plugs in the necks of 1 L. florence flasks containing 800 cc of this medium. After autoclaving, the medium in the first series of Erlenmeyer flasks was inoculated (sacs sterile), but in the second series the sacs were inoculated through the glass tubes (medium in flasks sterile). After inoculation the concentration of bacteria was 1 to 5 million per cc. Both series with duplicate controls not inoculated were incubated at 37°C for 18 hr. Diazo tests[6] were then performed on samples of medium from inside and outside of the sacs. Typical results are shown in Table I.

To determine if the increase in azo color were the result of PAB production, the drug medium in the sterile sac was tubed, inoculated with *Es. coli*, and its bacteriostatic potency was gauged.[5] Were PAB production the cause of the large increase in azo color, the new drug medium from the sterile sac would not induce bacteriostasis. However, it did so, indicating that the substance responsible for the additional azo color does not block sulfonamide action as does PAB.

Experiments were then conducted to gauge the influence on the synthesis of this amine of (a) the concentration of drug, (b) the size of the inoculum, (c) the added presence of PAB or other sulfonamide inhibitors. In brief, the results were as follows: (a) very little diazotizable material was produced when concentrations of drug insufficient for

TABLE I.
Production of Diazotizable Substance During Bacteriostasis with 2.09 mg% Sulfadiazine.

Sample	Vol. of medium, cc	Diazo conc. after incubation, mg%*	Diazo total, mg*	Amt sulfadiazine in medium, mg	Increment of new amine, mg*
Sac—inoculated	92	3.01	2.86		
Flask—sterile	797	2.38	19.00		
		Total	21.86	18.2	3.6
Sac—sterile	100	2.68	2.68		
Flask—inoculated	1015	2.91	29.55		
		Total	32.23	23.0	9.0

(*Es. coli* 1-5 million per cc in gelatin hydrolysate medium.)
*For convenience in expressing the results the new amine is assumed to produce as much color as an equimolar amount of sulfadiazine.

‡ Thanks are due Dr. Otto Bier of Sao Paulo, Brazil, for the very helpful suggestion that bacteria can be grown in water with the nutrients in cellophane sacs.

[6] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537. Colors read in the Klett-Summerson photoelectric colorimeter.

bacteriostasis were used; excessive drug concentrations¶ did not augment its production, (b) the amount formed varied directly with the total number of bacterial divisions, (c) PAB reduced the production of chromogen in direct, linear proportion to the PAB/Drug ratio used; addition of infusion broth to this medium prevented its formation (and also bacteriostasis).

The production of this diazotizable substance is intimately associated with bacteriostasis; it is also formed with bacteriostatic concentrations of sulfapyridine and sulfathiazole but its detection is difficult with the less potent sulfanilamide which requires far greater concentrations¶ for bacteriostasis.[5]

Isolation of the new substance is in progress. It is stable to boiling, dialyzable, and extractable with butyl alcohol.

Summary and Conclusions. During growth of *Es. coli* in synthetic medium with bacteriostatic concentrations of sulfonamide, a diazotizable substance is produced. This is not p-aminobenzoic acid; in fact, its production is prevented by those amounts of PAB necessary to block the action of sulfonamides. The substance is not produced in the absence of sulfonamide nor when bacteriostasis does not occur.

During bacteriostasis by sulfonamides in this medium there is a change in the metabolism of *Es. coli* so that a metabolite with a diazotizable grouping similar to the therapeutically active group of the drug is produced. Experiments are in progress to determine whether the production of this substance is specifically elicited by the drug or whether it is a normal metabolite which accumulates when a sulfonamide interferes with the growth of the organisms.

¶ Colorimetric differences are not accurately detectable when high concentrations of drug are tested, since the errors may exceed the increment sought.

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New Crystalline Forms of Tomato Bushy Stunt Virus.

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From the first report on the crystallization of tobacco mosaic virus[1] until recently, only 2 additional crystalline forms of purified viruses have been reported, the non-birefringent dodecahedra[2,3] of tomato bushy stunt virus (TBS) and the faintly birefringent plates of tobacco necrosis virus.[4] These were obtained by means of crystallization with electrolytes such as ammonium sulfate. A new crystallization technic for large proteins employing hydrophilic colloidal materials such as heparin, starch, etc., has resulted in the crystallization of a hitherto uncrystallized hemocyanin, the usual crystalline form of the anisotropic viruses such as tobacco mosaic virus, and new crystalline forms of TBS[5] and of a tobacco necrosis virus.[6]

In continuing studies on the crystallization of TBS by means of heparin and other substances, two previously unobserved crystalline forms of this virus were obtained. One form was obtained using heparin under conditions somewhat different from those reported previously. To a solution of TBS in

* Fellow in the Medical Sciences of the National Research Council.

1 Stanley, W. M., *Science*, 1935, **81**, 644.

2 Bawden, F. C., and Pirie, N. W., *Brit. J. Exp. Path.*, 1938, **19**, 251.

3 Stanley, W. M., *J. Biol. Chem.*, 1940, **135**, 437.

4 Pirie, N. W., Smith, K. M., Spooner, E. T. C., and McClement, W. D., *Parasitology*, 1938, **30**, 543.

5 Cohen, S. S., *J. Biol. Chem.*, 1942, **144**, 353.

6 Cohen, S. S., and Stanley, W. M., unpublished data.