diet or by raising the cholesterol content to about 1 per cent. That a high fat content in the diet favors the symptoms has previously been reported.[7]

These observations are interpreted by the assumption that certain regions of the capillary systems of the E-deficient chick are unable to withstand even the normal osmotic pressure of the blood and that the capillaries are easily damaged by histamine or an abnormally high supply of cholesterol as well as by other possible changes in the milieu with which the capillary wall is in contact.

The fact that fat and cholesterol favor the symptom suggested to us that it might be desirable to test the effect of some lipotropic substances.

Incorporation in the vitamin E deficient diet of 2 per cent. Lipocaic, a water-soluble preparation from pancreas (L. R. Dragstedt, *et al.*)[8] gave a high degree of protection against exudates even if the diet contained a relatively high amount of salts such as 7.2 per cent. of McCollum's salt mixture number 185. A chemical test showed that the effect of the lipocaic preparation could not be due to contamination with vitamin E. Inositol was then tested because Gavin and McHenry[9] have reported that this substance has a similar lipotropic effect as lipocaic. 1.5 per cent. of inositol in the diet was found to give a high degree of protection, whereas 1.1 per cent. of choline chloride was without any effect. Ineffective also was 5 per cent. gum arabic and 2 per cent. of acetone treated soy bean phosphatide was nearly ineffective.

This is believed to be the first instance where non-lipoid substances of animal origin have been found to counteract a symptom of vitamin E deficiency. An investigation as to whether these substances will also counteract other symptoms of lack of vitamin E, as well as a study of the protective factor in the lipocaic preparation and the way in which it acts, should be of importance in the elucidation of the mode of action of vitamin E.

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MECHANISM OF SULFONAMIDE ACTION. II. INHIBITION OF BACTERIAL RESPIRATION BY SULFANILAMIDE AND BY ITS INACTIVE ISOMERS

UNTIL very recently the only valid method for the study of sulfonamide action was based upon chemotherapeutic experiments, using animals infected with pathogenic bacteria. Recent investigations of the competitive inhibition of sulfonamides by *p*-aminobenzoic acid have shown that this antagonism can be made the basis of a suitable *in vitro* method.[1] The mechanism of the sulfonamide action is not clearly revealed by the *in vivo* experiments, and even the *in vitro* experiments based upon *p*-aminobenzoic acid antagonism involve the over-all process of bacterial growth in measuring sulfonamide activity. A recent report by Sevag *et al.*[2] attracted our attention because it was an attempt to study chemotherapeutic action of sulfonamides on a less intricate system. Our experiments using this method have convinced us that the inhibition of bacterial respiration by high concentrations of sulfonamides should not be regarded as typical sulfonamide action. We have found, for example, that the respiration of resting cells of *Escherichia coli*, *Staphylococcus aureus* or of *Streptococcus pyogenes* prepared after the manner of Sevag, is inhibited by the meta and ortho derivatives of amino benzenesulfonamide just as by sulfanilamide itself. The data in Table I show that of the two chemotherapeutically inactive isomers, the meta form behaves exactly as sulfanilamide, while the ortho form gives considerably more inhibition.

TABLE I

EFFECT OF .04 M SULFANILAMIDE AND ITS ISOMERS ON BACTERIAL RESPIRATION ON GLUCOSE IN M/60 PHOSPHATE BUFFER IN AIR

	Control		Sulfanilamide		Metanilamide		Orthanilamide	
pH	6.2	7.2	6.2	7.2	6.2	7.2	6.2	7.2
E. coli Q_{O_2}	37	38	25	24	26	25	12	11
Inhibition			32 per cent.	37 per cent.	30 per cent.	34 per cent.	67 per cent.	71 per cent.
Staph. aureus Q_{O_2}	63	51	41	38	39	35	23	30
Inhibition			35 per cent.	25 per cent.	38 per cent.	31 per cent.	63 per cent.	41 per cent.
Strep. pyogenes Q_{O_2}	55	50	44	36	44	36	35	29
Inhibition			20 per cent.	28 per cent.	20 per cent.	28 per cent.	36 per cent.	42 per cent.

E. coli is a typical fecal strain.
Staph. aureus is F.D.A. strain.
Strep. pyogenes is strain 1896 M obtained from Dr. J. S. Lockwood, University of Pennsylvania.

[7] H. Dam, J. Glavind, I. Prange and J. Ottesen, Royal Danish Academy of Science, *Biological Communications*, 16: 7, 1941.

[8] L. R. Dragstedt, C. Vermeulen, W. C. Goodpasture, P. B. Donovan and W. A. Geer, *Archives of Internal Medicine*, 64: 1017, 1939.

[9] G. Gavin and E. W. McHenry, *Jour. Biol. Chem.*, 139: 485, 1941.

[1] Orville Wyss, K. K. Grubaugh and F. C. Schmelkes, *Proc. Soc. Exp. Biol. and Med.*, 49: 618–622, 1942; H. M. Rose and C. L. Fox, SCIENCE, 95: 412–413, 1942; W. B. Wood, *Jour. Exp. Med.*, 75: 369–381, 1942.

[2] M. G. Sevag and M. Shelburne, *Jour. Bact.*, 43: 411–462, 1942.

In addition to showing that compounds totally devoid of chemotherapeutic activity (*i.e.*, true sulfonamide action) give inhibition equal to or exceeding that given by sulfanilamide, these data also show that the inhibition of the hemolytic streptococcus is not greater than that of the staphylococcus which generally is more resistant to sulfonamides.

Further, the effect upon respiration of a more active sulfonamide, sulfacetimide, was compared with that of sulfanilamide. This compound was selected because it would dissolve in .04 M concentrations. Preliminary experiments with sulfathiazole indicated that significant reductions of the rate of respiration could not be obtained with concentrations up to 100 mg per cent., the upper limit of solubility.

TABLE II

EFFECT OF .04 M SULFANILAMIDE AND SULFACETIMIDE ON RESPIRATION OF *E. coli* ON GLUCOSE IN M/60 PHOSPHATE BUFFER IN AIR

	Q_{O_2}		
	Control	.04 M Sulfanilamide	.04 M Sulfacetimide
pH 6.2	155	126	125
Inhibition ..		19 per cent.	19 per cent.
pH 7.2	143	126	121
Inhibition ..		12 per cent.	15 per cent.

Sulfacetimide shows no greater activity in this experiment than sulfanilamide. When 10 mg per cent. *p*-aminobenzoic acid was added to some of the flasks containing .04 M sulfonamide it did not reverse the inhibition of respiration by either sulfanilamide or sulfacetimide.

Finally an attempt was made to compare inhibition of respiration and of growth, using resistant organisms. The relative sulfonamide resistance of a parent strain of *E. coli* and a resistant strain developed from it is given in Table III.

TABLE III

CONCENTRATIONS OF SULFONAMIDES PERMITTING ONE-HALF MAXIMUM GROWTH RATE OF *E. coli* IN SYNTHETIC MEDIUM AT PH 7.0

	Parent Strain	Resistant Strain
Sulfanilamide ...	3.4 mg per cent.	62 mg per cent.
Sulfaguanidine ..	3.4	63
Sulfapyridine ...	.17	1.6
Sulfadiazine	.077	.34
Sulfathiazole	.073	.35

Inoculum = 100,000 cells per ml.

However, when the effect of .04 M sulfanilamide on the respiration of resting cells of these organisms was compared, equal inhibition was obtained with both strains.

TABLE IV

EFFECT OF .04 M SULFANILAMIDE ON GLUCOSE RESPIRATION OF RESISTANT AND NON-RESISTANT *E. coli* IN M/60 PHOSPHATE BUFFER, PH 7.2 IN AIR

	Control Q_{O_2}	.04 M Sulfanilamide Q_{O_2}
Parent strain	61	52
Inhibition		15 per cent.
Resistant strain ..	69	58
Inhibition		16 per cent.

SUMMARY

These data indicate that the inhibition of bacterial respiration is not a suitable criterion for the presence or absence of true sulfonamide activity.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

MICROMETER BURETTE

THE microburette recently described by Scholander[1] in common with all other modifications of a Rehberg burette[2] can only be used with solutions that do not attack mercury. Burettes of this type get dirty quickly, probably because grease creeps along the mercury. They are also hard to clean. To avoid these difficulties, we have constructed and used a burette combining a micrometer with a syringe, as has been done in one imported microburette. The anvil of a micrometer is cut off and a glass syringe mounted on a simple clamp in line with the spindle. Rubber bands attached to two hooks near the knurled head of the micrometer and to the plunger hold the latter tight against the spindle. A delivery tube can be attached to the syringe with a No. 0 one-hole rubber stopper; or if necessary a broken syringe of the same glass can be drawn out and fused on to the orifice of the syringe. A brass washer should be cemented to the outer end of the plunger and accurately perpendicular to its axis to act as a thrust bearing against the spindle. This bearing should be oiled occasionally. A convenient support can be made by screwing the yoke of the micrometer to the boss of a universal burette clamp, which can then be attached to a ring stand. The clamps on the syringe should be lined with friction tape to protect the glass and prevent slippage. Extra clamps permit the use of syringes of different sizes. We have used a 1-inch micrometer which delivers about 0.4 cc from a 1 cc tuberculin syringe or 1.5 cc from a standard 2 cc syringe. The syringes can

[1] P. F. Scholander, SCIENCE, 95: 177, 1942.
[2] P. B. Rehberg, *Biochem. Jour.*, 19: 270, 1925.