tablet (0.5 mg) of estradiol (Ovocylin, Ciba) per day. This routine was maintained without variation until September 14, 1940. In the 5 months spanned by this period, menstruation-like bleeding occurred 3 more times, on May 2, May 29, and on August 4. Pelvic examination again showed the uterus to be normal. There was no further show of blood up to September 14, at which time the dose of estrogen was unavoidably altered.

Three periods of uterine bleeding were observed, therefore, the intervals between which (after the first induced period) were 18, 28, and 68 days, plus 31 days without bleeding until the observations were terminated. Thus this patient bled 3 times on constant estrogen-dosage, although the "cycles" were not regular. This is equally apt to be so in monkeys (see Corner's My. No. 102, in which the cycles were 26, 25, 15, and 44 days ([1], page 247); see also Zuckerman's My. No. OM78 ([2], page 453) in which the cycles were 48, 43, 35, 46, 31, 40, and 41 days). Even so, the present case would appear to offer sufficient similarity to the monkey data to render it certain that in woman, too, menstruation-like bleeding may occur under suitable circumstances on constant dosage of estrogen. There is, accordingly, no known difference between the monkey and woman in the methods by which menstruation may be experimentally induced.

11822

Inhibition of Respiration of Dysentery Bacilli by Sulfapyridine.*

ALBERT DORFMAN, LESTER RICE, STEWART A. KOSER AND FELIX SAUNDERS.

From the Departments of Biochemistry and of Bacteriology and Parasitology, University of Chicago.

In a previous paper from this laboratory it has been shown that nicotinamide and some of its derivatives serve as essential growth factors for many dysentery bacilli.[1] More recently evidence has been presented which indicates that nicotinamide acts in some way other than, or in addition to, serving as a building block for one or both of the known phosphopyridine nucleotides.[2]

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The clinical use of nicotinic acid to control the nausea and vomiting resulting from the administration of sulfapyridine suggested to us the possible antagonism between sulfapyridine and nicotinamide.[3,4] After this work was under way, a paper by West and Coburn[5] appeared showing that sulfapyridine counteracted the growth-promoting activity of diphosphopyridine nucleotide (DPN) on *Staphylococcus aureus.*

Space does not permit a review of the literature concerned with the mechanism of action of sulfapyridine on microörganisms, but for the most part such work has concerned itself with the effect of sulfapyridine on the rate of multiplication of various microorganisms.[6] MacLeod[7] has shown that sulfapyridine inhibits the lactic, pyruvic, and glycerol dehydrogenases of sensitive strains of pneumococci. The ability to metabolize glucose was not affected.

A detailed description of the method of preparation of the cells used for the oxygen uptake measurements has been given elsewhere.[2] Briefly, the method consists of growing dysentery bacilli on a glucose containing synthetic medium deficient in nicotinamide. The cells are collected by centrifugation, washed twice with M/20 phosphate buffer (pH 7.4), and finally suspended in the phosphate buffer for use in oxygen uptake experiments. The direct method of Warburg is used for determining oxygen uptake.

It should be noted that the medium used in the respiration studies is free of nitrogen so that no chance of cell multiplication exists during the course of the experiments.

All tests were performed in duplicate and the essential experiments reported in this paper have been repeated several times. All comparisons of activity were made on the same suspension at the same time so that any variation in activity from day to day does not affect the results.

The dysentery culture used in these studies was of the Sonne type (strain 8).

Lederle sulfapyridine for standard solutions was used in all experiments.

It was found that the most consistent inhibitions were obtained

1 Dorfman, A., *et al., J. Inf. Dis.*, 1939, **65**, 163.

2 Saunders, F., Dorfman, A., and Koser, S. A., *J. Biol. Chem.*, in press.

3 Detweiler, H. K., *et al., Arch. Int. Med.*, 1940, **65**, 1144.

4 McGinity, A. P., *et al., J. M. A. Georgia*, 1939, **28**, 54.

5 West, R., and Coburn, A., *J. Exp. Med.*, 1940, **72**, 91.

6 *Clinical Uses of Sulfanilamide and Sulfapyridine and Allied Compounds*, Long, P. H., and Bliss, E. A., The Macmillan Company, New York, 1939.

7 MacLeod, C. M., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 215.

TABLE I.
Effect of Sulfapyridine on Nicotinamide-Stimulated Respiration of Dysentery Bacilli.

	O_2 uptake for 2 hr minus blank (A), cmm O_2	% inhibition
A. 1.6 cc cell suspension	36.1	
B. 1.6 cc cell suspension + 0.8[2] nicotinamide in vessel	217.8	
C. 1.6 cc cell suspension + 0.8[2] nicotinamide in vessel incubated 1 hr, room temp. 800 γ sulfapyridine tipped in after incubation	188.7	13.3
D. 1.6 cc cell suspension. 0.8[2] nicotinamide tipped in after 1 hr incubation, room temp.	199.5	
E. 1.6 cc cell suspension + 800 γ sulfapyridine in vessel incubated 1 hr, room temp. 0.8[2] nicotinamide tipped in after incubation	26.7	86.6

0.3 cc of 2% glucose solution added to all vessels and all made up to a final volume of 2.7 cc.

if 800 γ of sulfapyridine were used in each vessel (30 mg %), and if the cells were allowed to stand with the sulfapyridine at room temperature for 1 hour before the nicotinamide was tipped in from the side-arm. The routine procedure adopted was to put the vessels and manometers in the bath exactly 50 minutes after the cells and sulfapyridine were mixed. After a 10-minute equilibration the stopcocks on the manometers were closed and the material from the side-arm was tipped into the main part of the vessel.

Table I is illustrative of a typical experiment. The oxygen uptake figures given are averages of duplicate determinations. Combination A is the blank, that is, the respiration of the deficient cells without the addition of nicotinamide. Numerous experiments have shown that this residual respiration is not inhibited by the addition of sulfapyridine. Combination B illustrates the effect of the addition of nicotinamide to deficient cells. It will be noted that the respiration is markedly increased. In this case the nicotinamide is added to the vessel together with the cells prior to incubation. The addition of sulfapyridine after incubation with nicotinamide (combination C) results in a small inhibition, which is just outside the range of experimental error. This inhibition varied from about 8 to 15% in different experiments.

Combination D again illustrates the stimulation due to nicotinamide, but in contrast to B the nicotinamide is tipped in after the incubation period. It will be noted that the oxygen uptake of D is somewhat lower than that of B. This decrease in activity on incubation has always been observed and is probably due to the gradual

inactivation of the free enzyme during the period of incubation. The previous addition of nicotinamide seems to protect the enzyme. Combination E illustrates the effect of sulfapyridine when it is added before the nicotinamide. It will be noted that the inhibition is very marked in this case. Under these conditions the inhibition has always been striking although the percentage inhibition has varied from 80 to 95% in different experiments. This consistency was not obtained with smaller concentrations of sulfapyridine and without the period of incubation.

Table I strikingly illustrates the apparent antagonism between the action of nicotinamide and sulfapyridine. Experiments are now in progress to determine quantitatively the nature of this antagonism.

The results shown in Table I raise the question of the effect of simultaneous addition of sulfapyridine and nicotinamide. The results of numerous experiments on this point show a wide variation, but on the whole there seems to be a definite inhibition with a value between that obtained in C and E in Table I.

When sodium lactate is used as substrate in the Warburg vessels results qualitatively similar to those obtained with glucose are obtained. The percentage inhibition is somewhat smaller with lactate as substrate than with glucose.

Respiration stimulated by DPN is inhibited by sulfapyridine in a manner which is at least qualitatively similar to the inhibition illustrated in Table I.

These results strongly indicate that the action of sulfapyridine on microörganisms may be related to the role of nicotinamide in their metabolism.

Summary. It has been found that sulfapyridine in concentrations of 30 mg % inhibits the stimulation of respiration due to nicotinamide. This inhibition is apparently competitive since the sulfapyridine must be added prior to the addition of the nicotinamide to obtain this effect. While percentage inhibition has varied in different experiments, sufficient experimental data have been obtained so as to leave no doubt as to the validity of the inhibition.