tions limiting growth. The effect varies in magnitude with different vitamins, and is especially pronounced with pyridoxine and with folic acid. The relative effectiveness of concentrates of the latter in promoting growth of *L. casei* may differ considerably when determined on 2 media which differ only in their alanine content.

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Effect of Sulfonamides on Coenzyme I-Linked Enzyme Systems.*

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At present the best theory for the mode of action of the sulfonamides postulates their inhibition of certain metabolic reactions due to the similarity in structure of the sulfonamides and the prosthetic groups of the respiratory enzymes. Evidence in support of this theory has been contributed by studies on the inhibition of the respiration and growth of bacteria, and also by the discovery that p-aminobenzoic acid has a counteracting effect on sulfonamides. Particular emphasis has been placed on the reactions involving coenzyme I, because its active group, nicotinamide, is chemically similar to the pyridine ring of sulfapyridine and to the isosteric ring of sulfathiazole. This work has been summarized by Dorfman and Koser[1] in a paper on the inhibitory effect of sulfapyridine and sulfathiazole on the nicotinamide stimulated respiration of the *dysentery bacillus*. Since all of the previous work has dealt with respiration in the whole cells of bacteria, an attempt was made to reproduce this effect in isolated enzyme systems.

For this purpose, reactions were employed in which coenzyme I was necessary for maximum activity, and where a quantitative relationship could possibly be traced between the amount of coenzyme I needed for stimulation, and the amount of sulfa drug needed for inhibition. The first reaction tried was that of the yeast fermentation method for the quantitative determination of coenzyme I.[2,3,4] The method used was essentially that of Axelrod and Elvehjem,[4] except that it was found necessary to add 0.1 mg muscle adenylic acid and 20 μg cocarboxylase to each respirometer flask for maximum carbon dioxide evolution. Coenzyme I was used at levels of 5 and 10 μg per flask.

Sulfapyridine or sulfathiazole in solution as its sodium salt (10 mg per cc) was added to the flasks with the rest of the constituents before the addition of the yeast preparation. Amounts added varied from 1-10 mg per flask.

With the final pH of the solution in the flask adjusted to 6.2-6.6 so that the normal fermentation of the yeast was not inhibited by the alkaline reaction of the sodium sulfonamides, no inhibition occurred even at the highest level of the drug. The use of the hexose diphosphate at twice the normal level, with the coenzyme I at 5 μg or 10 μg per flask did not alter the results.

Another approach to the problem was the use of enzymes present in normal rat liver which require coenzyme I in their hydrogen-transport systems. One such system—malic

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oxidase—has been studied by Potter.[5] The livers were obtained from healthy stock rats weighing from 200-250 g. The reaction was followed by measurement of the oxygen uptake in a Warburg respirometer at 37°. Each flask contained: M/4 phosphate buffer (pH 7.6), 0.4 cc; M/2 malate, 0.2 cc; M/2 glutamate, 0.2 cc; cytochrome *c*, 4×10^{-4} M, 0.1 cc; coenzyme I, 5 mg per cc (50% pure), 0.3 cc; M/10 nicotinamide, 0.3 cc; 2% liver homogenate[6] in water, 1.0 cc; and water to make 3 cc. 0.3 cc 10% NaOH was placed in the center well of each flask to absorb the carbon dioxide evolved. Glutamate was added to aminate the oxalacetate formed, which would otherwise have inhibited the reaction; nicotinamide was added to help prevent the immediate destruction of coenzyme I. The sodium salt of sulfapyridine or sulfathiazole was added to the flasks in amounts varying from 3-10 mg. Succinylsulfathiazole was also tested at 0.4 mg per flask.

In no case did the Q_{O_2} vary significantly from the control value.

Obviously, experiments could not be limited to systems containing coenzyme I as a hydrogen carrier, since the sulfonamides might also inhibit other metabolic reactions. Hence, the effect of sulfapyridine, sulfathiazole, and also succinylsulfathiazole was tried in the succinoxidase system.[7,8] A procedure similar to that employed for malic oxidase was followed. Each flask contained the following: M/4 phosphate buffer (pH 7.4), 0.4 cc; M/2 succinate, 0.3 cc; cytochrome *c*, 4×10^{-4} M, 0.1 cc; Ca^{++} and Al^{+++} as chlorides, 0.012 M, 0.1 cc each; 0.5 cc 2% liver homogenate in water; and water to make 3 cc. The sodium salt of sulfapyridine or sulfathiazole was added in amounts varying from 1-5 mg; succinylsulfathiazole was added to the limit of its solubility, 0.5 mg per flask.

It was evident from the data obtained that the results of these experiments were also completely negative and that none of these compounds affected the activity of succinoxidase.

Sulfapyridine, sulfathiazole, and succinylsulfathiazole also had no effect on the respiration of rat liver in the presence of pyruvate or fumarate in simple systems containing only homogenate, substrate and buffer, but with coenzyme I and nicotinamide added to the fumarate system.

An attempt was also made to determine if administration of large doses of a sulfonamide to a living animal would have any effect upon the respiration of its tissues. When doses of 0.25-0.40 g of sodium sulfapyridine were given orally or intraperitoneally to normal, healthy stock animals (6 rats) 3-6 hours before death, or daily for several days, the activity of the systems was not altered from the normal values. The ranges of Q_{O_2} values, as determined under our conditions, on many normal animals, are as follows: succinate, 80-100; malate, 60-75; pyruvate, 8-14; fumarate, 9-16.

Discussion. It is unlikely that p-aminobenzoic acid had any effect in the present studies. The tissue samples would contain about 0.1 μg of p-aminobenzoic acid and the other constituents of the reaction mixtures would contribute only minute amounts. One part of p-aminobenzoic acid counteracts less than 1000 parts of sulfapyridine or sulfathiazole in *in vitro* bacterial growth studies. Since 1000 to 5000 μg of the sulfa drug were added in our experiments, the ratio of drug to p-aminobenzoic acid was probably too high for counteraction to take place. Furthermore, sulfapyridine or sulfathiazole presumably may interfere with nicotinamide coenzymes in such a manner that p-aminobenzoic acid is not involved. Dorfman and Koser[1] have found that p-aminobenzoic acid has no effect on sulfapyridine inhibition of resting cell respiration of *dysentery bacilli* but that nicotinamide or coenzyme I can reverse this effect.

The results obtained in the present studies add to the increasing evidence that sulfapyridine or sulfathiazole interfere with the production and/or utilization of nicotinamide rather than with the function of the pyridine

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nucleotides themselves. McIlwain[9] has reported that pyridine 3-sulfonic acid inhibits the conversion of nicotinic acid to nicotinamide by *Staph. aureus*. Teply, Axelrod, and Elvehjem[10] found that nicotinamide, nicotinamide-ribose nucleoside and coenzyme I were of about equal potency in partially counteracting sulfapyridine bacteriostasis of *L. arabinosus*. Considerably larger amounts of nicotinic acid had no effect. The fact that both Teply *et al.*[10] and Dorfman and Koser[1] found larger amounts of coenzyme I to be necessary in the presence of sulfapyridine than under normal conditions does not necessarily invalidate the theory that the drug does not interfere with the function of the coenzyme I molecule. It is possible that coenzyme I is broken down before it is taken in by certain bacterial cells. It is also possible that in the respiration of intact cells, coenzyme I is continually broken down and sulfapyridine or sulfathiazole interferes with its resynthesis. This latter viewpoint is considerably strengthened by the work of Morel[11] who found that coenzyme I disappears from Proteus cells metabolizing pyruvate, and its content can be maintained if nicotinamide is supplied. She concludes that the coenzymes "wear out" while functioning only if there is an occasional irreversible change of the nicotinamide.

Summary. Our results indicate that sulfonamides do not interfere directly with the functioning of coenzyme I in yeast fermentation or in several systems in normal rat liver. The available experimental evidence does suggest the possibility that the drugs may inhibit the synthesis of the coenzymes.

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Structural Particularities of Antifibromatogenic Steroids: Experiments with Δ^4-androstene-3,17-dione and Cholestenone.*†

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The senior writer has called attention to the fact that the naturally occurring steroids which prevent abdominal fibroids induced by estrogens in the female guinea pig were all Δ^4-3-keto-steroids,[1] as progesterone, desoxycorticosterone, dehydrocorticosterone, and testosterone. There was apparently a parallelism between the progestational and antifibromatogenic activities of these substances; the antifibromatogenic activity of progesterone was greatly superior to that of testosterone propionate[7] or of Δ^{16}-dehydroprogesterone which was not antifibromatogenic.[3] The question arises how other Δ^4-3-keto-steroids known to have little or no progestational activity might behave when administered simultaneously with fibromatogenic quantities of estrogens. Two naturally not occurring

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