

the same activity as streptothricin against *B. subtilis*, *A. aerogenes*, and *P. vulgaris*; it was less active against *S. aureus* and certain strains of *Salmonella*; it was much more active against *B. mycoides*, *B. cereus*, *Mycobacterium phlei*, *Serratia marcescens*, *Ps. aeruginosa*, *Ps. fluorescens*, and *Cl. butylicum*.

Streptomycin, like streptothricin, possesses strong bactericidal properties, and preliminary experiments tended to indicate that the two substances are also comparable in their low toxicity to animals and in their *in vivo* activity. The various chemical and biological properties of streptomycin tend to point to this compound as one closely related to streptothricin; the fact that it differs from the latter in the nature of its antibacterial activity

may indicate a closely related but not the same type of molecule.

Summary. A new antibacterial substance, designated as streptomycin, was isolated from two strains of an actinomyces related to an organism described as *Actinomyces griseus*. This substance resembles streptothricin in its solubility in water, mode of isolation and concentration from culture medium, its selective activity against gram-negative bacteria, and its limited toxicity to animals. However, the two substances differ in the nature of their respective bacteriostatic spectra as well as in their quantitative action upon different bacteria. It is suggested that one is dealing here with two closely related chemical compounds.

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Production of Riboflavin Deficiency in the Monkey.*

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In a recent report from this laboratory¹ it was demonstrated that the rhesus monkey (*Macaca mulatta*) grows well and remains in excellent health for at least 14 months on a highly purified diet consisting of sucrose 73, purified casein[†] 18, salts 4, corn oil 3, cod liver oil 2, and adequate quantities of the pure vitamins together with a "folic acid" concentrate. Through the use of this diet we have attempted to produce specific vitamin deficiencies by withholding the vitamin concerned from the daily supplement. The "folic acid" concentrate (norite eluate according to

the method of Hutchings *et al.*²) was assayed for its content of each of the B group of vitamins and found to contain between 1 and 2 γ of riboflavin per gram equivalent of original "solubilized liver extract"[‡] from which the concentrate was made. The maximum riboflavin furnished by a 5% level of the concentrate was therefore 10 γ . Negligible quantities of riboflavin were contained in the purified casein and in the sucrose used in the diet.

The monkeys were given the basal diet *ad libitum* and all the vitamins except riboflavin were supplied in the daily supplement according to the procedure previously described.³ Recently obtained monkeys placed on the deficient diet showed growth failure after 6 to 8 weeks while other monkeys

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¹ Waisman, H. A., and Elvehjem, C. A., *J. Nutrition*, 1943, **26**, 361.

[†] Smaco vitamin-free casein, obtained from SMA Corp., Chagrin Falls, Ohio.

² Hutchings, B. L., Bohonos, N., and Peterson, W. H., *J. Biol. Chem.*, 1941, **141**, 521.

[‡] Obtained through the courtesy of Wilson Laboratories, Chicago, Illinois.

³ Waisman, H. A., Rasmussen, A. F., Jr., Elvehjem, C. A., and Clark, P. F., *J. Nutrition*, 1943, **26**, 205.



FIG. 1 AND 2.
The "freckled dermatitis" of riboflavin-deficient monkeys.

previously fed various diets containing liver, showed no deficiency until after longer periods of time. In contrast to thiamine-deficient animals, the riboflavin-low monkeys continued to eat and even in advanced deficiency marked anorexia was never observed.

The more significant signs of riboflavin deficiency developed gradually; a "freckled" type of dermatitis appeared first on the face (Fig. 1 and 2) and in the area of the groin; the small, red, dry spots became increasingly prominent and enlarged until they appeared as dark scabs covering nearly the whole area. In more severe cases of the "freckled dermatitis" other areas of the body, notably the arms and legs, became encrusted. The dermatitis occurred at the site of hair growth on the arms and legs, and none was seen at the hairless joints of the fingers and toes. This was in contrast to the presence of dermatitis of the face where a minimum of hair is found.

A well defined incoordination of the limbs was observed in those monkeys which were

maintained on the deficient diet for periods as long as 2 to 3 months. The grasping reflex was faulty and the strength both of arms and legs was diminished in 2 of the monkeys.

Some slight variation in the time of onset and in the severity of the symptoms was encountered in the individual animals. In most of the monkeys the first visible sign of the deficiency was the "freckled dermatitis." The deficient animals were pale and apathetic.

Hemoglobin determinations and red and white cell counts showed unmistakable changes from the normal blood picture of monkeys.³ Table I shows the blood values for 6 of the animals before the development of the deficiency and during the height of the syndrome; the sharp drop in the hemoglobin level was evident shortly after the appearance of the dermatitis. The time at which the lowest values were recorded varied for the different animals from 59 to 108 days after they were placed on the riboflavin-low diet. Another distinctive feature of the deficiency

TABLE I.

Monkey No.	Hemoglobin		Red blood cells		White blood cells	
	Normal value	During deficiency g	Normal value	During deficiency millions	Normal value	During deficiency thousands
53	13.4	4.9	5.5	2.0	11.0	8.9
57	14.6	9.7	5.8	2.7	14.0	6.6
64	13.9	6.3	5.28	2.4	13.2	6.42
80	14.9	4.0	4.58	2.3	13.9	7.0
81	13.0	7.72	6.1	3.02	11.0	6.08
82	12.9	8.0	6.09	1.7	13.8	11.8

was the reduced red cell count which usually preceded the decrease in hemoglobin. The white cell count was more variable, but definitely lower than normal values.

Administration of 50 μ g riboflavin per day to the deficient animals brought about remission of the early and milder cases of "freckled dermatitis" within 2 to 3 weeks. When the symptoms were of a more advanced nature, however, 50 γ riboflavin per day for 30 or more days were inadequate to give improvement of the animals but higher levels of 100 to 500 γ per day gave remission of all observed signs after 10 to 14 days. The dermatitis cleared rapidly, and "new looking" skin appeared on the hands, feet, and face after several weeks of riboflavin administration.

The time of reappearance of the symptoms after riboflavin was again discontinued was directly related to the level and duration of the administered dosage. When the animals were fed a supplement of 50 γ per day for 3 weeks, they were protected from dermatitis for the following 21 days, while the administration of 500 γ per day to the same animal for 21 days gave protection for approximately 70 days. The deficiency was produced several times in the individual monkeys and each time the animal responded to riboflavin therapy. It has been shown by several groups of workers that the greater portion of the administered vitamins are rapidly excreted and this finding undoubtedly accounts for the disproportionately shorter protective duration with the larger dosage.

Further discussion on the requirement of riboflavin must be deferred until experiments now in progress are completed. Since the diet contained no added biotin it was necessary to ascertain if the dermatitis might be related

in part to the secondary biotin deficiency. Accordingly, biotin as a crude concentrate and also in crystalline form were fed both before the onset of the deficiency and during the height of the syndrome with no effect either on the dermatitis or the blood picture. In more recent trials, the riboflavin deficiency signs have been produced in monkeys on diets containing added biotin from the beginning of the experimental period.

It is of interest that no cheilosis or vascularization of the cornea has been observed grossly in these riboflavin-deficient animals. Although 2 of the animals were kept on the low riboflavin diet for 5 and 7 months no readily apparent change in the cornea was observed. The acute type of deficiency produced here is perhaps quite unlike the more chronic and sub-clinical deficiency noted in humans.^{4,5,6,7} It should also be recognized that the riboflavin deficiency described in these monkeys is uncomplicated by lack of other vitamins as far as we are able to determine.

Summary. An acute riboflavin deficiency has been produced in monkeys fed a highly purified diet containing all known vitamins except riboflavin. A "freckled dermatitis" and an anemia are the most striking signs of the deficiency syndrome.

⁴ Oden, J. W., Oden, L. H., Jr., and Sebrell, W. H., *U. S. Public Health Rep.*, 1939, **54**, 790.

⁵ Kruse, H. D., Sydenstricker, V. P., Sebrell, W. H., and Cleckley, H. M., *U. S. Public Health Rep.*, 1940, **55**, 157.

⁶ Sydenstricker, V. P., Sebrell, W. H., Cleckley, H. M., and Kruse, H. D., *J. A. M. A.*, 1940, **114**, 2437.

⁷ Sebrell, W. H., and Butler, R. E., *U. S. Public Health Rep.*, 1938, **53**, 2282; *Ibid.*, 1939, **54**, 2121.