were prepared from cultures grown by the method of Dolman (Can. J. Pub. Health, **27**, 489, 1936) and were heat-treated to destroy alpha and beta toxins. The filtrates were applied to the sciatic nerve of the frog, *Rana pipiens*, after the nerve had been removed from the animal together with the attached gastrocnemius muscle. The muscle was secured between a muscle clamp and the recording arm of a kymograph, leaving one end of the nerve free for application of filtrate.

Application of a toxic filtrate to the free end of the nerve created an immediate reaction which caused peaks to be produced on an otherwise straight line recorded on the smoked drum of the kymograph. Nontoxic filtrates produced only the straight line effect.

All of the 93 strains of *S. aureus* had, at the time of isolation, met the biochemical criteria for pathogenic staphylococci. After nine months in stock culture only seven strains still met these criteria. This group of seven, as well as the five strains with a history of food poisoning and a record of having produced symptoms in monkeys, reacted positively by the kymographic technique. Eighty-six strains of *S. aureus* and one strain of *S. albus* were negative. Likewise, the sterile medium, its components, and Ringer's solution for cold-blooded animals gave negative reactions.

The filtrates created a reaction only when they were applied to the nerve. Bathing the muscle tissue with filtrate produced no response. Once a nerve-muscle preparation had been mounted on the kymograph, it could be used a number of times as long as it was bathed thoroughly between tests with Ringer's solution.

Tetanus toxin, used for control purposes, produced results on the kymograph identical to those obtained with staphylococcal filtrates. Whether the neurotoxic substance present in *S. aureus* filtrates is identical to gastroenterotoxin has not been established, but the symptomatology of staphylococcal food poisoning strongly suggests involvement of the nervous system. A detailed account of the procedure employed in the kymographic technique is soon to be published.

A COBALT-CONTAINING MEDIUM FOR SPORULATION OF STREPTOMYCES SPECIES

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A considerable number of different agar media, some quite complex, have been described for use in inducing sporulation in *Streptomyces* species. One of the simpler formulas, known as Bennett's medium, was described in a paper by Jones (J. Bact., **57**, 141, 1949). This has been found to be a rather generally use-

ful substrate in our experience, but sporulation on it by some *Streptomyces* species was found to be slow and sparse.

A number of modifications of Bennett's medium were investigated. The use of Amidex,[1] a dextrin, in place of glucose appeared to be of some advantage, but further addition of a low level of cobalt chloride greatly improved the rate and extent of sporulation of *Streptomyces fradiae*, strain 3535 (Waksman), which was being studied at the time. The inclusion of zinc and iron in place of cobalt appeared to suppress sporulation.

The basal medium employed consisted of 1 per cent Amidex,[1] 0.2 per cent N-Z Amine A[2] (an enzymatic hydrolyzate of casein), 0.1 per cent Difco beef extract, 0.1 per cent Difco yeast extract, and 2 per cent agar. Results with this medium and with inorganic supplements are given in table 1. Cotton-plugged test tubes, 16 by 150 mm, were employed. Media were autoclaved for 30 minutes

TABLE 1

Sporulation of Streptomyces fradiae, strain 3535, on several agar formulas

MEDIUM	PER CENT OF SURFACE GROWTH SPORULATED (VISUAL)*			
	5 ml slopes		10 ml slopes	
	6 days	10 days	6 days	10 days
Basal	50	70	30	35
Basal + 0.1 mg % $ZnSO_4 \cdot 7H_2O$	35	40–50	20	15–20
Basal + 2 mg % $CoCl_2 \cdot 6H_2O$	75	90–95	50	90
Basal + 2 mg % $FeSO_4 \cdot 7H_2O$	30	30–40	20	15–20
Basal + 20 mg % $FeSO_4 \cdot 7H_2O$	5	5	2	2–3

* Sporulation occurred in areas or zones fairly easily estimated. The values given are approximations and are not intended as precise figures.

at 121 C, cooled to harden, and held overnight in a 37 C ventilated incubator to dry the agar surface. Slopes of 5 and 10 ml of medium per tube were employed, and incubation of the inoculated slopes was at 28 C.

The generally better sporulation on the 5 ml slopes might be related to its drier surface. The advantage of the inclusion of cobalt in the substrate appears evident; therefore, the medium finally employed consisted of the basal formula described, supplemented with 2 mg per cent of $CoCl_2 \cdot 6H_2O$.

Utilization of this medium for the cultivation of a considerable number of *Streptomyces* species has shown that sporulation is generally as good as or better than is found on Bennett's medium. Pigmentation produced on this substrate by many organisms is quite pronounced, hence it is a potentially useful medium for the study of this important diagnostic character in the identification of *Streptomyces* species.

[1] Corn Products Refining Co., Argo, Illinois.

[2] Sheffield Farms Co., Inc., New York 19, N. Y.