things of importance along the trails of science for the direct or ultimate benefit of the public whose advancement is the eventual proof of the value of research. In the happy fusion of science, research, and management that forms the body of thought and action in the Institute, there is constantly optimistic realism in coping with common needs and in moving forward in discovery and improved practice. The investigational philosophy of the organization has as its frame of reference all the knowledge about research and its administration accumulated during the three decades in which the Institute's principles have been in successful application.—*William A. Hamor* (Mellon Institute of Industrial Research).

In the Laboratory

Determination of Penicillin K by Partition Chromatography

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The commercial penicillin produced since May 1944 has been shown to be less effective in the treatment of early syphilis than that made available prior to this date (*1, 2*). Coincident with this trend of decreased efficacy was the development of new strains of *Penicillium notatum* and *Penicillium chrysogenum* for increasing the commercial output of active material. The existence of four penicillins, X, G, F, and K, is known (*3*). Recently it has been recognized that the increased output has resulted in the production of proportionately greater amounts of penicillin K in the commercial product at the expense of penicillin G, which had predominated in the original commercial penicillin. Under the direction of the OSRD, studies were made of the four penicillins. It was shown that penicillin G was therapeutically active in the treatment of syphilis and that results obtained with commercial penicillin produced before 1944 approximated the results obtained with crystalline G (*2*). Moreover, it was demonstrated that penicillin K was therapeutically deficient in the treatment of syphilis, and it was concluded that the reduced efficiency of the commercial products marketed after May 1944 was probably due to the content of K (*2*). Thus, it became of paramount and immediate importance to develop some means of determining the quantitative amount of penicillin K in commercial samples.

Other investigators have utilized partition chromatography for the isolation of pure crystalline penicillins. This, together with the apparent variations in the hydrophilic properties of the penicillins, turned our attention to partition chromatography. A considerable number of solvents and eluents were investigated along with buffers of varying pH in order to ascertain the optimum conditions for resolving a mixture of the penicillins. This laboratory has successfully separated and quantitatively determined penicillin K in the presence of the other penicillins by means of partition chromatography. Because of the wide interest in this problem and the general urgency for such a method, it was deemed advisable to present an early report on our findings.

The following conditions have been successfully used for concentrations of 25–55 mg. of total penicillins: Twenty-five grams of silica gel prepared by the Gordon, Martin, and Synge technique (*4*) were thoroughly macerated with 12.5–16.5 ml. of 20 per cent potassium phosphate, with a buffer pH of 6.4. The quantity of buffer was varied somewhat depending on the adsorbability of the silica gel used. Washed chloroform was added to the mixture, and the resulting slurry was poured into a glass cylinder of 22 mm. inside diameter. Slight pressure was required to facilitate the uniform settling of the silica gel. The column was then used in a 10° C. room. The penicillins to be passed through the column were extracted into chloroform from pH 2.0 buffer at 0° C. Aliquots were assayed to determine the total amount of penicillin in the extract. When pure penicillins were used, each type was extracted and assayed separately. Aliquots of the extracts were mixed, passed through the column, and followed with sufficient cold chloroform to furnish two 50-ml. aliquots of eluate. Anesthesia ether saturated with water was used as eluent from this point on. The small amount of ethanol in this grade of ether probably aids in the subsequent elution. Twenty-five-ml. fractions were collected, and the progress of the chromatographic resolution was followed by means of an iodometric assay (*5*) of each fraction. In addition, differential assays were performed on enough fractions to identify and ascertain the sequence of elution of the penicillins. The latter assays were

obtained through the cooperation of the Division of Penicillin Control and Immunology. No activity appeared in the fractions until the major portion of chloroform was removed from the column. Fortuitously, the first band of activity to appear is that of penicillin K, in accord with its property of being the least hydrophilic of the four known penicillins. The total penicillin K was removed in three to four 25-ml. fractions, 70 to 75 per cent of the total appearing in one 25-ml. fraction. Subsequently, three to four 25-ml. fractions exhibiting no activity were collected prior to the appearance of the next active band. This provides for considerable latitude of operation. Elutions with ether were continued until all of the penicillin on the column was quantitatively recovered. The rate of elution was varied between 10 and 35 minutes per 25 ml. (with and without applied pressure) without causing any discernible differences.

From this laboratory's experience any losses entailed in the chloroform extraction are of equal degree for each of the penicillins. Consequently, the recovery of penicillin in the first band eluted from the column divided by the total amount of penicillin in the chloroform extract furnishes the per cent of penicillin K. To date this work has been confined to separating penicillin K quantitatively from the other penicillins.

A commercial sample of penicillins was subjected to the above procedure and a band of activity separated which was identified by differential assay as penicillin K.

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Letters to the Editor

Sulfa Drugs and the Treatment of Furunculosis in Trout

Furunculosis, probably the most feared disease of salmonoid fishes, is due to a general infection by a specific bacterium, *Bacterium salmonicida* Lehmann and Neumann. In this country it affects chiefly trout in hatcheries, but in Europe it is also very destructive of wild trout and salmon.

There has been no curative treatment or means of preventing its spread through any pond, pool, or trough of fish among which it has appeared.

In August 1945, when the disease appeared among fingerling brook trout (*Salvelinus fontinalis*) at Leetown, West Virginia, the writer was assigned the problem of finding a cure by means of sulfa or other drugs.

For this attempt, all the fish in the pool in which furunculosis (bacteriologically confirmed by D. F. W. Hachtel) was present were distributed among 15 lower troughs in one corner of the hatchery.

From another pool, brook trout of similar age and size, but believed to be free from furunculosis, were placed in a group of 5 troughs at the opposite end of the hatchery. The weight of fish per trough was 15–16 pounds, and the estimated number of fish per trough, 450–480. In each block or group of 5 adjacent troughs, 4 treatments and a control (with no medication) were assigned to troughs by random selection.

The original treatments, begun on 30 August 1945, consisted in administering sulfamerazine, sulfathiazole, and furacin (or 2–20–99, a new drug) by mixing them with the food, and in adding furacin to the water of the troughs. On and after 14 September sulfanilamide (in food) and sulfadiazine (in food) were substituted for the furacin treatments, which seemed less promising than sulfonamide treatments.

The assigned or approximate drug rate or dosage was the same for all lots, except that the rate for furacin was half that for the sulfonamides. That rate varied from 5 to 11.5 grams/day/100 pounds of fish.

Among the fish believed to be free from furunculosis, losses were negligible until gill disease appeared among them, and other of the experimental fish, on 23 September.

Among the fish from the lots from the infected pool, mortalities through 21 September were approximately as follows: no medication, 50 per cent; sulfamerazine, 17 per cent; sulfathiazole, 23 per cent; furacin in food followed by sulfanilamide, 28 per cent; and furacin in water followed by sulfadiazine, 40 per cent.

Among the three infected lots being treated with sulfamerazine, mortality sharply declined within a week; and after 11 September (the 13th day of treatment) there were only three deaths until the gill disease appeared, after 23 September. Sulfathiazole seemed definitely helpful, but the mortality dropped less rapidly and had not completely stopped on 23 September. According to analysis of variance, the superiority of results with sulfamerazine was highly significant.