at the low temperature contain a high level of toxin in their blood, which disappears after they become normal on transfer to the high temperature. The reverse is also true. DDT-treated roaches apparently normal at 35° C contain blood that will not excite the isolated central nervous system, but roaches from the same group rendered prostrate with DDT-poisoning by transfer to 15° C contain blood that will produce a high level of stimulation in the isolated nerve cord.

At present we are studying the nature of the toxin produced in the blood of DDT-poisoned roaches, the role of temperature in relation to the disappearance of the toxin, and the presence of the toxin in relation to the mechanism of action of DDT. The results of these studies will be reported in greater detail later.

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Differentiation between Circulins A and B and Polymyxins A and E by Paper Chromatography

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Circulin, a mixture of antibiotics produced by *Bacillus circulans* Q-19, consists of basic polypeptides that are biologically and chemically closely related to the polymyxins (*1–7*). In fact, Peterson and Reineke (*2*) reported that the circulin fraction that they studied most intensively (since then designated as circulin A) had qualitatively the same composition as polymyxins A and E. All three antibiotics were thought to contain L-threonine, D-leucine, L-α, γ-diaminobutyric acid (DABA), and an optically active isomer of pelargonic acid with the properties of 6-methyloctanoic acid (*8*). Unlike polymyxin A, however, circulin was inactivated in the presence of lipase. These workers had no polymyxin E available for comparative work and were therefore unable to rule out the possibility that circulin A and polymyxin E were the same.

[1] Many colleagues gave us invaluable help in this work, and we gladly express our thanks to the following: George Brownlee, formerly of the Wellcome Research Laboratories, Beckenham, Kent, Eng., for sending us a sample of polymyxins A and E, and for arranging with Tudor Jones to compare circulin and polymyxin E by paper chromatography; and Harold Nash, of the Pitman-Moore Company, Indianapolis, Ind., for his continuous cooperation and permission to use some of his unpublished data in this paper.

Peterson and Reineke obtained circulin A after repeated chromatography over a mixture of equal amounts of Darco G-60 and Celite 545, using 25% aqueous tertiary butanol adjusted to pH 4.0, with sulfuric acid as the developing solution. This system separated crude circulin into two main components, namely, fraction A and the more rapidly moving circulin B. Using a combination of the above procedure and paper chromatography with the system to be described later, we obtained preliminary evidence that strain Q-19 probably produces, in addition to the two major components already mentioned, at least three other ninhydrin-positive, biologically active entities of as yet unknown nature. This paper records the fact that circulins A and B can be distinguished from polymyxins A and E by paper chromatography.

Preparation of the circulins. Separation of circulin A from circulin B was accomplished by the procedure mentioned above, or by the following method suggested by Nash (*9*): Impure circulin sulfate was dissolved in a minimal amount of *n*-butanol that had been saturated with a 0.1 *M* sodium citrate–hydrochloric acid buffer (pH 2). This solution was added to a column of Celite 545, which had been moistened by the buffer saturated with *n*-butanol. The developing agent was *n*-butanol saturated with buffer. The fractions collected were extracted twice with 10-ml portions of distilled water. After the biological potency of each extract was determined (*cf.*[*1*]), the appropriate extracts were pooled and concentrated *in vacuo* to a small volume. An acetone solution of picric acid was used to precipitate the biologically active material from an aqueous solution. The picrate was converted to the hydrochloride by gaseous hydrogen chloride or concentrated hydrochloric acid.

Polymyxins A, D, and E. Polymyxin A was approximately 48% pure, and its hydrochloride assayed 4800 polymyxin A u/mg. Polymyxin E regarded as essentially pure was furnished in the form of a base, and its sulfate assayed 11,600 polymyxin E u/mg before it was converted to the free base with gaseous ammonia at pH 8.2. Polymyxin D was used as hydrochloride and contained 1280 polymyxin D u/mg.

Paper chromatography. The antibiotics (usually 100 μg in 5 μl) were applied to Whatman No. 1 filter paper strips and permitted to dry. Unless otherwise indicated, the solvent system used consisted of the following: 49.5% *n*-butanol, 49.5% water, and 1.0% glacial acetic acid (*9*). The strips were hung for descending chromatography in an airtight glass cylinder and were allowed to equilibrate for about 2 hr with the vapors from the aqueous phase of the solvent system. The nonaqueous phase was then used to develop the chromatogram. Development continued for at least 60 hr. Ninhydrin was used to indicate the position of the peptides. Duplicate strips served to make certain that the ninhydrin-positive materials were actually antibiotically active. After chromatography the strips were placed for 8 min on the surface of an agar medium that had been seeded with *Escherichia coli ATCC 26* (*cf.* [*1*]), and then removed.

Following growth of the organism, the locations of the zones of inhibition were compared with those of the ninhydrin-positive areas.

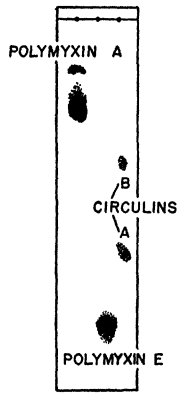

FIG. 1. Descending paper chromatogram of polymyxin A, polymyxin E, and a mixture of circulin A and B. Water, *n*-butanol, and acetic acid were used as the developing solution. In this system, in contrast to the one used by Peterson and Reineke (*2*), circulin A moves more rapidly than circulin B.

Fig. 1 shows a chromatogram of polymyxin A, polymyxin E, and an impure sample of circulin, containing both A and B, which assayed 5800 u/mg. The antibiotics were present as hydrochlorides. This figure shows that neither circulin A nor B is chromatographically identical to polymyxins A or E.

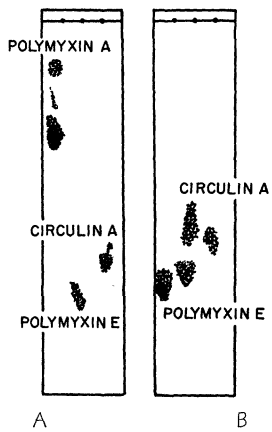

FIG. 2. Separation of polymyxin A, polymyxin E, and circulin A by descending paper chromatography. In *A* the antibiotics were applied singly, whereas in *B* single antibiotics were compared to a mixture of polymyxin E and circulin A.

Fig. 2, *A* illustrates a typical chromatogram of polymyxin A, polymyxin E, and circulin A (all as hydrochlorides). As can be seen, circulin A moved at a different rate than either polymyxin A or polymyxin E. It was possible to separate a mixture of the three antibiotics just as readily, although the individual components in a mixture sometimes moved at slightly different rates than they did when applied singly. Fig. 2, *B* shows a chromatogram of polymyxin E, a mixture of E and circulin A, and circulin A alone. The result again indicates that circulin A and polymyxin E are distinct entities.[2]

According to preliminary evidence obtained by Nash (*9*), who used the paper chromatographic procedure that he developed and that was described above, hydrolysates of circulins A and B contain isoleucine in addition to the constituents found to be present by Peterson and Reineke (*2*). Although we were able to confirm these observations, final proof for the presence of isoleucine will have to await actual isolation and identification of isoleucine or one of its derivatives.

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[2] A private communication from George Brownlee to one of us (H. K.) stated that Tudor Jones, of the Wellcome Research Laboratories, Beckenham, Kent, Eng., also was able to demonstrate different rates of mobility for a relatively impure sample of circulin and polymyxin E under the conditions which he used to prepare paper chromatograms.

Colloidal Graphite in the Preparation of Samples for Gas-Flow Counting

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In tracer studies the sensitivity of methods involving isotopic dilution is a function of (1) the activity of the original preparation, (2) the sensitivity of the counting technique, and (3) the accuracy of the counting technique. Frequently the activity of the original material is limited, either by possible physiological effects of irradiation or by unavailability of highly active material. The sensitivity of β-ray counting is greatest where the material is introduced into the counting chamber, either as a gas or as a nonvolatile solid. A convenient method for accomplishing this is found in the use of the gas-flow counter (*1*), in which the sample is introduced as a solid into the counter and a stream of counter gas is flushed constantly through the chamber (Fig. 1). With relatively low sample masses, this method gives up to 50% β-ray counting efficiency.

In the application of this technique, a serious disadvantage experienced in several laboratories, including our own, has been the inherent inaccuracy of counting, as evidenced by nonreproducibility of counting rates on the same sample when counted on different days: most of the time the results would not check within statistical expectations. Even a C^{14}-polystyrene