bison of the Neo-Indian period is thought to have evolved gradually from *occidentalis*. Probably the bison remains were incorrectly identified. Smaller individual variants of *occidentalis* are nearly inseparable from some of the larger individuals of plains and woodland bison.

Should the Agate Basin bison remains prove to be of a fossil species, the archeologist, when he finds fossil bison remains (particularly of the *antiquus* subspecies), can assert with reason that any associated cultural remains belong to the Paleo-Indian period. When modern bison are found, he can attribute the associated cultural remains to the Neo-Indian period. He will however, have to be cautious with *occidentalis* remains because of the similarity of this form to the surviving race.

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29 August 1955

Quantitative Infrared Spectroscopy of Desoxyribonucleic Acid in the Fractional Milligram Range

Since desoxyribonucleic acid (DNA) is generally considered as the material from which genes are made, its characteristic infrared spectrum is of great biological interest (*1, 2*). Because of the very slight solubility of DNA in any but aqueous solvents and the rather strong absorption of infrared rays by water, it is desirable to examine the material in the solid state. However, the solid preparations of DNA, such as powders, films, or Nujol pastes, that are used for this purpose require relatively large amounts of substance and give only qualitative spectroscopic data at best.

This paper (*3*) describes quantitative infrared spectroscopy of DNA in the fractional milligram range with use of suspensions in solid KBr, which is very transparent to infrared, for specimen preparation and a condensed infrared beam for spectroscopic examination. The technique developed for this purpose principally follows the KBr-disk procedures that have been used mainly for qualitative infrared spectroscopy (*4–6*). Various improvements of the procedure, especially of the technique of specimen preparation, however, have enabled us to carry out quantitative infrared spectrometry in the fractional milligram range.

Aqueous solutions of highly polymerized DNA (Worthington) were mixed with dilute solutions of KBr of highest purity (Merck-Darmstadt) to give exact amounts (20 to 300 μg) of DNA in 10 ml of 1.2-percent KBr. The mixtures were frozen quickly in a Dry Ice-acetone bath and then lyophilized with a modified cryochem freeze dryer. The freeze-drying cycle lasted for 24 to 30 hours, and the drying was completed at 25° to 30°C and 30 to 90 μ-Hg pressure.

Fifty milligrams of the frozen, dried material was transferred into a steel die of ¼-in. diameter. The die was evacuated to about 1 mm-Hg and transparent disks of 1-mm thickness were pressed with a Carver press. The optimum time of pressing was 1 minute. The optimum pressure corresponded to the reading of 8000 lb/in.^{2} on the Carver press gage. Single or double plunger dies were used. The single plunger dies had to be greased with a minute quantity of graphite in order to prevent cracks when the disks were pressed out. The double plunger die was relativly simple to use and allowed pressing of disks of almost identical weights (50 mg) and thicknesses (1 mm).

Infrared spectroscopy was carried out with a Beckman I.R. 2 spectrometer that was equipped with a beam-condensing unit comprised of a system of silver chloride lenses. The disk was inserted in a disk holder, focused in the condensed beam, and examined spectroscopically. Measurement of bands was carried out by the conventional base-line method.

The infrared spectra of the disks given in Fig. 1 show all the characteristic vibrations of DNA that have previously been observed in the powders, films, or pastes. Many important vibrations, such as the weak band at 9.80 μ, which is present in DNA but absent in ribonucleic acid, appear at least as clear or clearer in the disk spectrum from less than 50 μg DNA than they were found in the spectra from powders, films, or pastes requiring 20 times more material.

The evaluation of our method for quantitative infrared spectrometry of small amounts of DNA was carried out in disks containing 9, 18, 27, 36, 54, and 72 μg of DNA. Three different absorption bands—namely, the bands at about 8.1 μ, 9.80 μ, and 10.30 μ—were examined in each disk, and the absorbancies found with the base-line method were plotted versus concentrations (Fig. 2).

Figure 2 demonstrates the linearity of the function of absorbancy versus concentration found in all the three absorption bands and indicates strict observance of Beer's law at even low DNA concentrations. Furthermore, the linearity of these functions is shown by agreement of the three extinction coefficients that were calculated from the experimental data. At DNA concentrations of 36 μg and above, extinction coefficients for all the three bands show percent average deviations of ±3 to ±4.2 percent. At DNA concentrations below 36 μg, the deviations increase in the two weaker bands (9.80 μ and 10.30 μ). In the strongest band (8.1 μ), however, the percent average deviation of the extinction coefficients remains at ±3 percent even at DNA concentration as low as 18 μg.

It is believed that the method for quantitative infrared spectrometry of small

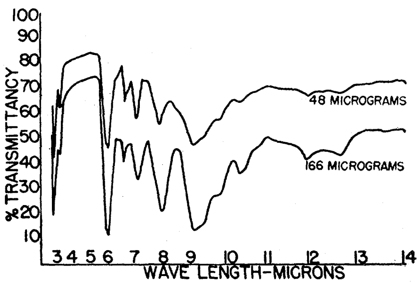

Fig. 1. Infrared spectra of desoxyribonucleic acid suspended in solid potassium bromide. The upper curve gives the transmittancy of a disk, containing 48 μg of DNA in 50 mg of KBr; the lower curve gives the transmittancy of a disk containing 166 μg of DNA in 50 mg of KBr.

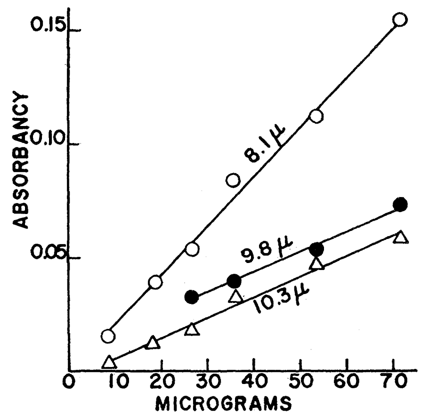

Fig. 2. Graphic indication of infrared absorbancy versus concentration of DNA in disks containing 9, 18, 27, 36, 54 and 72 μg of DNA. The upper curve was obtained from measurements of the bands at 8.1 μ, the middle curve from the absorbancies of the bands at 9.80 μ, and the bottom curve from those of the bands at 10.30 μ.

amounts of DNA shown in this paper will be important for biological and structural studies and that further experiments with smaller dies will permit infrared analysis of even smaller quantities.

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8 August 1955

Directional Differences in Pigeon Homing

The results of pigeon homing investigations made at the Max-Planck-Institut at Wilhelmshaven were the first to suggest a directional difference in orientation. The data from a number of releases showed a definite superiority of the homing results when the pigeons were taken south for release compared with the results when they were taken east. This was true for distances as short as 15 mi, but it was also true for 100 and more mi. Results from north and west releases were not available for a direct comparison.

Further work was done in the Carolinas (*1*). To collect data bearing more explicitly on the question, we made pigeon releases simultaneously from four points north, east, south, and west of the loft. Releases of single birds were made at each point of the "cross" release pattern at intervals of 10 min. Observers at the loft recorded the time of arrival of each bird. In the present report, we base the comparison of performance from the four directions of flight entirely on homing success.

A series of 12 cross releases was made with birds from two different lofts in the Durham region. In November 1954, pigeons from a loft located in the Duke Forest were flown twice at a distance of 16 mi and a third time at a distance of 53 to 60 mi. Birds from a second loft, which was located on the edge of Durham, were used in the period from February to April 1955 in a series of eight cross releases at 17¼ mi and in a ninth release from 53 to 59 mi (*2*). In the 16- to 17-mi flights, we selected release points that would provide a symmetrical cross pattern of release points and therefore could not choose places that were suitable for observing departure orientation. Forestry lookout towers were used as release points for the 53- to 60-mi flights; the cross pattern was not perfectly symmetrical. However, the distances for the flights from the north and from the south remained equal.

Within each series, the intervals between releases varied from 3 to 15 days; the same birds were used repeatedly, new ones being introduced to replace losses. On successive release days, the direction of displacement of individual birds was shifted to limit practice effects. In the series of releases at 17¼ mi, the whole groups were shifted, the sequence for the birds that were first displaced to the north being N, E, W, S, N, S, E, W; the others were rotated in step with these. For the other releases, new groups were formed—for example, birds that had last been sent north were assigned equally to groups that were to be taken E, S, and W. About half the birds were inexperienced in that they had never been removed forcibly from the loft area before they were used in the cross releases; the remainder had received a few previous homing releases from various directions. For their first cross release, the birds were always assigned to a group going in a different direction from that of their immediately preceding displacement.

Despite the 10-min release interval between birds, flight pairs or larger groups were formed on the way home in some cases. To meet the requirement of statistical independence in the data, we have considered only those birds that arrived singly and the fastest member of each group in the analysis of the results. The cross releases provided homing records for 558 of the short-distance flights and for 117 of the 53- to 60-mi flights. These were approximately equally distributed at each distance among the four directions. Figure 1 summarizes the homing performances for both distances separately. Birds displaced to the south yielded a relatively larger number of returns at 15 mi/hr or faster. Likewise, flights from the south showed a remarkably low number of losses. Birds displaced to the north, on the other hand, made the smallest number of quick returns and showed by far the largest number of losses. Chi-square tests of the data in Fig. 1 show significant departures from the distribution expected by random sampling (*3*). The winds, which averaged about 8 mi/hr and were most often from the west, did not appreciably affect the results. Flights from the north were slightly favored over those from the south by the average wind direction.

The fact that directional differences are found at only 16 to 17 mi strongly suggests that even at this short distance orientation is not primarily based on landmarks. Sheer landmark orientation or random searching should result in comparable homing performance from all directions. However, even with the practice afforded by eight flights from the 17¼-mi distance (twice from each point for each group), the north-south contrast in homing apparently was not erased (*4*).

There is no reasonable ground for doubting that a south-to-north superiority of homing exists in these birds as far as these two loft locations are concerned. It should be noted that the two Durham lofts are situated 2.6 mi apart, which makes it unlikely that individual features of the loft site are responsible for the observed effect. Since further work is

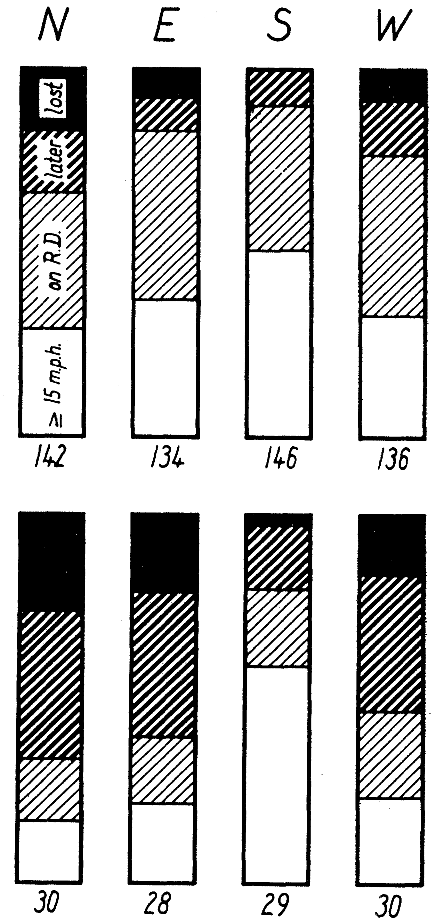

Fig. 1. Percentage differences in homing success dependent on displacement directions (indicated on top). (Top) 16 to 17¼ mi; (bottom) 53 to 60 mi. The figures beneath the columns show the number of independent flights. Legend: (white) percentage of returns at 15 mi/hr or faster; (fine hatching) percentage of returns slower than 15 mi/hr but within release day; (heavy hatching) percentage of returns on subsequent days; (black) percentage of birds lost.