there is evidence that serum proteins resulting from alcohol fractionation are denatured to the extent that, although still soluble, they are poorly metabolized when they are injected into an animal (*4*, pp. 460–461). Only the salting out method by the use of neutral salts is thought to leave the protein fractions in their native, unaltered form (*4*, p. 173) and apparently only by this method was Eagle able to obtain fractions that were consistent in their activity.

The possibility that specific proteins are essential for the activities of at least some mammalian cells would also seem compatible with some of the properties and roles that have been described for certain proteins. They could act on the cell membrane and affect its permeability to other materials. There is evidence that the protein hormone insulin acts in this way (*5*). There is good evidence that certain plasma proteins can enter cells fairly readily (*6*); and, once inside the cell, the essential protein may act by complexing smaller compounds whose activity may depend on this action. The tremendous importance of this property of proteins and the dependance of the biological function of many compounds on the alteration of properties resulting from such complex formation has been pointed out in detail by Needham (*7*). It seems to be an accepted fact that protein hormones somehow alter the metabolism of the cells they affect; and, because some proteins are present in relative abundance, this should not in itself rule out an essential metabolic role for them.

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22 November 1955

## Early Man and Fossil Bison

At certain sites in and near the Great Plains, there are associations of fossil bison and early types of projectile points. Correlating these associations, I have developed a possible sequence of certain projectile points and of contemporaneous fossil bison forms (Fig. 1) (*1*).

In 1947, Skinner and Kaisen enumerated the known localities where fossil bison are represented (*2*). At a number of these localities, Folsom, Plainview, and Scottsbluff points have been found. Since 1947, Scottsbluff points have also been identified at the Finley site in association with *Bison* (*Bison*) *occidentalis* (*3*).

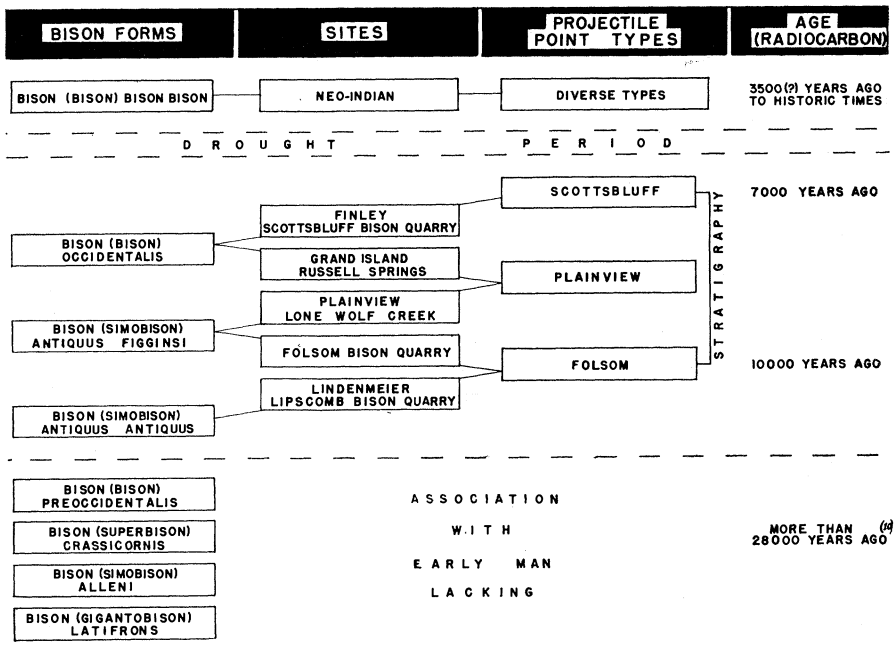


Fig. 1. Associations of bison forms with sites and projectile point types in the Great Plains.

Excavations at the MacHaffie site revealed that the Folsom complex is older than the Scottsbluff (*4*). This fact has been confirmed elsewhere by radiocarbon dates on a Folsom site (9883 ± 350 years ago) and on a Scottsbluff site (6876 ± 250 years ago)—dates that indicate an age difference of some 3000 years (*5*).

The Folsom-Scottsbluff succession having been established, it is evident that Fig. 1 shows the best possible way of correlating the fossil bison and projectile points.

The number of sites with associations will have to be increased before the results of the correlation can be regarded as established fact. Nevertheless, until contradictory evidence comes to light, it provides a working hypothesis.

Sound contradictory evidence is lacking. At the Lime Creek site, a Scottsbluff layer was reportedly found stratigraphically below a layer attributed to the Plainview complex (*6*). However, according to Krieger (who originally conceptualized the Plainview point), the Lime Creek specimens are not Plainview (*7*).

Plainview points may prove to be younger than Scottsbluff on the basis of a supposed geologic relationship between the Lime Creek site, with its Scottsbluff layer, and the nearby Red Smoke site, where authentic Plainview points were found. The geologic evidence, however, has not yet been published in a convincing manner.

One potential flaw in the chain of evidence must be noted. The points from Grand Island and Russell Springs are not typical Plainview points. Probably they are merely variants of the Plainview type. At the Red Smoke site, these so-called Meserve points were found in the same cultural layer as typical Plainview points.

Early man (Paleo-Indian) occupations appear to be separated from the later (Neo-Indian) occupations of the plains by a drouth period of some 3000 years, beginning about 6500 to 7000 years ago. Fossil forms of bison have not yet been found in any of the known Neo-Indian sites. And, with one possible exception, modern bison have not been found in sites attributed to early man on the Great Plains.

The possible exception is the Agate Basin site in Wyoming (*8*). There, the bison (said to be of historic species) were not classified according to the system Skinner and Kaisen developed. Unfortunately, they were discarded from the U.S. National Museum because they appeared to be only a superfluous addition to an already adequate collection of modern bison (*9*).

A renewed excavation of this site and a reexamination of its bison remains will show whether or not the historic species lived contemporaneously with *occidentalis* in the Paleo-Indian period. Contemporaneity seems to be out of the question, because the historic plains

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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### References and Notes

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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

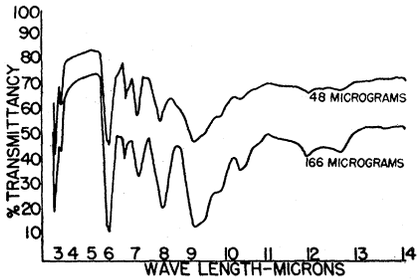


Fig. 1. Infrared spectra of desoxyribonucleic acid suspended in solid potassium bromide. The upper curve gives the transmittancy of a disk, containing 48 μg of DNA in 50 mg of KBr; the lower curve gives the transmittancy of a disk containing 166 μg of DNA in 50 mg of KBr.

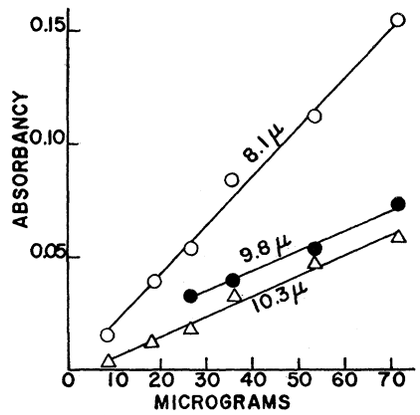


Fig. 2. Graphic indication of infrared absorbancy versus concentration of DNA in disks containing 9, 18, 27, 36, 54 and 72 μg of DNA. The upper curve was obtained from measurements of the bands at 8.1 μ, the middle curve from the absorbancies of the bands at 9.80 μ, and the bottom curve from those of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