Table 1. Control measurements

No.	Sample	Net counts/min
P-40	Modern young poplar from Haibak	6.40 ± 0.19
P-40a		6.34 ± 0.15
		Average 6.37 ± 0.12
P-46	C-1, culture I	6.63 ± 0.15
P-46a		6.89 ± 0.13
		Average 6.76 ± 0.10 (modern)

II and in certain respects also comparable to the numerous flint pieces collected on the surface of the slope outside the shelter.

The samples for carbon-14 dating were taken from fire-pits in the different levels and consisted of charcoal intermixed with some earth. They were all treated with hydrochloric acid to remove inorganic carbon compounds before they were processed. The samples listed in Tables 1 and 2 with prefix P were dated in our laboratory. The older ones were beyond the range of our counting method (*1*), and three of them were dated by Hans E. Suess. These three samples are listed with prefix W (*2*). With our present equipment, the maximum range is 25,000 years; the range of Suess's equipment is 32,000 years (*3*). In some cases a single sample is listed as "older than" two different dates; these are not inconsistent, but are the limits of the two different counting methods. These dates should never be quoted without the prefix "older than."

The measurements obtained with samples P-40, P-40a and P-46, P-46a (Table 1) are in agreement with previous measurements of modern carbon from other parts of the world. The ages have been calculated on the basis of a value of 6.70 ± 0.10 counts per minute for the modern net counting rate (*1*).

Table 2. Radiocarbon dates

No.	Sample	Age (yr)
P-54	B-1, culture I	2740 ± 300
P-53	A-4, typologically culture I	10,580 ± 720
P-48	C-8, top of culture III (small sample	20,000 + ∞ − 5000
P-42, P-42a	A-5, typologically culture III	Older than 25,000
W-224	A-5, typologically culture III	34,000 ± 3000
P-50, P-50a	B-9, culture III	Older than 25,000
W-226	B-9, culture III	Older than 32,000
W-225	C-11, culture III	Older than 32,000
P-51	C-12, culture III	Older than 25,000
P-49	C-13, culture III	Older than 25,000

The date of sample P-53, 10,580 ± 720 years (Table 2), probably fits culture I; culture II has no charcoal; culture III may be dated by samples W-224, W-225, and W-226 in the mid-30,000's before the present or earlier. It is worthy of note that these dates make an Upper Paleolithic blade culture, at least, contemporaneous with a Middle Paleolithic culture, that of Haua Fteah in Libya, Suess's sample W-85, which dated 34,000 ± 2800 years or possibly older (*4*).

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20 June 1955

Association of Molybdenum with Nitrate Reductase from Soybean Leaves

The nitrate reductases from soybean (*Glycine max.* Merr.) leaves (*1*), *Neurospora crassa* (*2*), *Escherichia coli* (*3*), and soybean nodules (*4*) are similar in many of their characteristics. All are flavoproteins that catalyze the oxidation of reduced pyridine nucleotides by nitrate to yield nitrite and oxidized coenzymes. The various nitrate reductases are similarly inhibited by KCN, NaN_3, and sulfhydryl complexing agents but have different specificities for reduced coenzymes and different *p*H optima. It has been demonstrated that the reductases from *Neurospora* (*5*) and *Escherichia coli* (*3*) are specifically activated by molybdenum. The purpose of this communication is to indicate that this metal also is associated with the nitrate reductase from soybean leaves.

An experiment was conducted to determine whether or not Mo^{99} applied to soybean seedlings was incorporated into the nitrate reductase of the leaves. Soybean seeds (variety Ogden) were germinated in flats of sand, as has been previously described (*1*). Four days after the seeds were planted the flats were treated with a sufficient amount of a solution containing 3.5 mc of Mo^{99} per gram of MoO_3 (dissolved in dilute NH_4OH and neutralized with HCl) to obtain a concentration of 10 ppm total Mo in the sand. When the seedlings were 10 days old, leaves were harvested, fractionated and assayed for nitrate reductase, as has been previously described, using DPNH as a hydrogen donor (*1*). An aliquot from each fraction was ashed in a muffle furnace and counted for radioactivity with a Geiger-Müller counter.

The nitrate reductase activities and radioactivities of the various fractions expressed on a protein basis (*6*) are reported in Table 1. The enzyme activity and radioactivity of the first two fractions were not closely correlated; however, as the purity of the enzyme increased in fractions III and IV, Mo^{99} was definitely associated with the enzyme activity of the fractions. The results of a second experiment showed the same trends as those indicated in Table 1. Further purification of the enzyme would be necessary before a constant ratio of enzyme activity to radioactivity could be expected.

In order to obtain further evidence concerning the metal requirements of this enzyme, $(NH_4)_2SO_4$ precipitates (fraction III) were prepared from fresh leaves or acetone powders of leaves, as was previously described (*1*). The enzyme samples were dialyzed for 5 hours against 0.25*M* purified phosphate buffer (*p*H 7.0) containing 0.001*M* concentrations of both glutathione and KCN, as is described by Nicholas and Nason (*5*). Afterward the samples were dialyzed for 8 hours against a similar buffer solution containing glutathione but

Table 1. Association of radio molybdenum with nitrate reductase during purification. The purification procedure was identical with that described previously (*1*), with the exception that a 0 to 50 percent $(NH_4)_2SO_4$ precipitate was obtained in fraction III instead of a 0 to 40 percent precipitate

Fraction	Protein (mg/ml)	Enzyme activity* (units†)	Radioactivity (counts/sec†)
I Crude extract	3.00	9.4	8.9
II First gel eluate	0.33	189.2	10.5
III 0 to 50 percent $(NH_4)_2SO_4$ precipitate	0.52	368.0	12.7
IIIA Supernatant from III	0.34	6.4	5.0
IV Second gel eluate	0.19	1006.6	30.0
IVA Residue after second gel adsorption	0.20	8.0	6.0

* One unit of enzyme activity is defined as the amount required to produce 1×10^{-3} μM of nitrite in 5 minutes, using the standard assay (*1*).
† Expressed per milligram of protein.

no KCN. Aliquots of the dialyzed enzyme containing 0.05 mg of protein were tested for nitrate reductase activity using DPNH in the assay (*1*) and purified buffer and KNO_3 (*7*). Other aliquots of the enzyme solution were preincubated at 2°C with 0.01 μM of various metals, and then were assayed for enzyme activity using purified reagents. The mean increases or decreases in activity in percentages from the addition of the various metal salts were: $FeSO_4$, +15; Na_2MoO_4, +47; $FeCl_3$, +15; $MnSO_4$, +4; $CuSO_4$, −43; and $ZnSO_4$, −2 for two experiments. Dialysis of the enzyme against buffers at *p*H value below 7.0 failed to remove Mo from the enzyme. These samples, however, consistently showed a small increase in activity from the addition of $FeCl_3$ or $FeSO_4$.

In view of the results reported here, it seems apparent that Mo is a constituent of the soybean leaf nitrate reductase. The results suggest that iron may partially replace Mo as a metal factor in the enzyme (*8*).

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20 June 1955

Comparative Tonographic Study of Normotensive Eyes of White and Negro Persons

Opinion is divided concerning a predilection of Negroes for glaucoma. The pertinent literature is reviewed in our extensive publication (*1*).

More pigment being present in the Negro eye, pigment granules may more readily be deposited in the trabecular meshwork, in the canal of Schlemm, and in its outlets, causing an obstacle to aqueous outflow.

The purpose of the work reported here (*2*) was to determine, with the aid of an electronic tonometer, whether there are any differences in intraocular pressure (IOP), facility of outflow (c), and production of aqueous humor (K), between

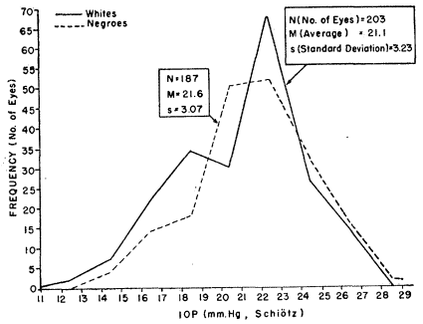

Fig. 1. Intraocular pressure distribution in 203 eyes of white persons and 187 eyes of Negro persons.

normotensive eyes of white and Negro persons. In other words, we have looked for a possible difference in the aqueous humor dynamics of Caucasians and Negroes.

One hundred seven white persons, representing a total of 203 eyes, and 97 Negro subjects (187 eyes) were studied. Of the white subjects, 52 were female (100 eyes) and 55 were male (103 eyes). Of the Negro subjects, 45 were female (86 eyes) and 52 were male (101 eyes). All persons were between 40 and 92 years of age. All eyes were free of manifest ocular disease.

It was, of course, impossible to determine the percentage of Negro blood in our subjects. We did take the precaution of selecting subjects with very dark skins, because their eyes are richer in pigment. We believe that our colored examinees are representative of the typical American Negro.

We performed tonography on each eye, using a Mueller electronic tonometer without recording attachment. The technique used was the standard one described by various authors; we followed all the precautions recently outlined by Stepanik (*3*). The values were recorded every 30 seconds; the total duration of each tonometry was 4 minutes. The facility of outflow was calculated from the formula

$$c = \frac{V_\Delta}{(\mathrm{Av}\, P_t - P_0)\, t},$$

and the rate of production of aqueous humor was calculated from the formula

$$K = P_0 \times c.$$

For interpretation of these formulas, the reader is referred to Grant's original papers (*4*).

All our data were evaluated statistically, using the F test of Snedecor and the t test of Student. First we compared the values of intraocular pressure, facility of outflow, and production of aqueous humor of the eyes of the white and Negro persons studied. Figures 1, 2, and 3 show the curves of distribution for intraocular pressure, facility of outflow, and production of aqueous humor, respectively, of these two groups with the relative average values (M) and the standard deviations (s). By comparing our data with the appropriate values from the F and t tables, we found that the difference between the samples was attributable to chance. In other words, no significant difference between intraocular pressure, facility of outflow, and production of aqueous humor of normotensive eyes of white and Negro persons exists.

The same statistical analysis was made on the values of intraocular pressure, facility of outflow, and production of aqueous humor for the eyes of white and Negro men and women. The calculations and the values for the F and t tests proved that all data were homogeneous for both female and male groups. Intraocular pressure, facility of outflow, and production of aqueous humor of the eyes of Negro women and men do not differ significantly from those of the eyes of white women and men.

Our investigations proved that (i) there is no significant difference between intraocular pressure of normotensive eyes of Negro and of white adults; (ii) the facility of outflow of aqueous humor is not significantly lower in the Negro eye than it is in the white eye; and (iii) the production of aqueous humor is the same in the eyes of members of both races.

Since the resistance to outflow is the reciprocal of the facility, we conclude that the eyes of Negroes do not present more resistance to outflow than do the eyes of Caucasians. The eyes of persons of the white and Negro races differ ana-

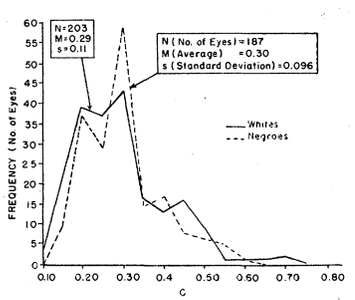

Fig. 2. Facility of outflow in the same eyes.

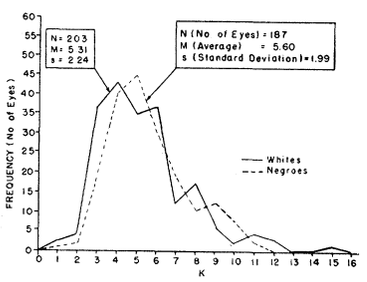

Fig. 3. Production of aqueous humor in the same eyes.