TABLE 1

Product counted	Av atomic number
Glucosazone	3.96
Bisphenylhydrazone mesoxalaldehyde	4.1
Aldomedone	3.5
Formaldimedone	3.5
($BaCO_3$	17)

by us in this step only occasionally had this value. In the majority of cases their melting point was 123°–125° C. We could not, therefore, duplicate this step.

It is clear, therefore, that the step in Aronoff and Vernon's technique of obtaining the C-1 + C-2 value by degrading bisphenylhydrazone of mesoxalaldehyde with alcoholic potash is not dependable. Until more is known about the products of this reaction, the technique of Aronoff and Vernon, as proposed by these authors, cannot be used.

The other steps of their method, however, have been found satisfactory, and because of the advantages of this method we did not wish to drop it. The method, as claimed by the authors, is rapid, and the various products, with the exception of glyoxalosazone, are easily obtained and plated.

In a search for some other method of precipitating and counting C-1 + C-2, we decided to isolate these two carbons as acetaldehyde and precipitate it with dimedone (*4*). Such a precipitation is quantitative and rapid, and the crystals are readily plated in pyridine. The technique for glucose degradation becomes now a combination of some of the steps of Wood, Lifson, and Lorber (*1*) and some of those of Aronoff and Vernon (*2*), plus a new step involving precipitation of acetaldehyde as aldomedone. Fig. 1 presents the scheme of such a degradation.

Glucose is fermented with *L. casei,* and the lactic acid formed is oxidized with $KMnO_4$. Acetaldehyde produced from C-1 + C-2 or C-6 + C-5 is precipitated with dimedone and counted as such. Glucosazone, bisphenylhydrazone mesoxalaldehyde, and formaldehyde are produced and counted according to Aronoff and Vernon. Table 1 gives the average atomic number of various compounds counted. Since this number is about the same for all the compounds counted, the backscattering effects are similar. For this reason the activities of such compounds are directly comparable.

TABLE 2

DISTRIBUTION OF C^{14} IN VARIOUS POSITIONS OF GLUCOSE AS PERCENTAGE OF TOTAL ACTIVITY

Glucose sample	Degradation technique used	Carbon atoms of glucose		
		3 and 4	2 and 5	1 and 6
1	Wood, Lifson, and Lorber	34.5	32.5	33
1	Vittorio, Krotkov, and Reed	33.7	32.1	34.2
2	Wood, Lifson, and Lorber	52	25.9	22.1
2	Vittorio, Krotkov, and Reed	50.9	26.1	23.0

Two samples of glucose, with uniform and nonuniform distribution of C^{14} in various positions, were degraded using both our proposed modification and the original scheme of Wood, Lifson, and Lorber. The results are given in Table 2, which shows that the results obtained by both methods agree within about 2.5%.

References

1. WOOD, H. G., LIFSON, N., and LORBER, U. *J. Biol. Chem.,* **159**, 474 (1945).
2. ARONOFF, S., and VERNON, L. *Arch. Biochem.,* **28**, (3), 424 (1950).
3. DIELS, O., MEYER, R., and ONNEN, O. *Ann.,* **525**, 94 (1936).
4. YOE, J. H., and REID, L. C. *Ind. Eng. Chem.,* Anal. Ed., **13**, (4), 238 (1941).

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Competition of the Aliesterase in Rat Serum with the Pseudo Cholinesterase for Diisopropyl Fluorophosphonate[1]

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The molar concentration of pseudo cholinesterase in certain mammalian sera can be determined by the use of a competitive reversible inhibitor, Nu-683, the dimethyl carbamate of (2-hydroxy-5-phenylbenzyl) trimethylammonium bromide, and an analog of prostigmine (*1*).

The concentration of inhibitor that causes 50% inhibition of the pseudo-cholinesterase activity (I_{50}) is determined at various concentrations of serum; a plot of I_{50} against the relative enzyme concentration should give a straight line with a slope equal to one half the molar concentration of pseudo cholinesterase in the serum solutions. Typical results of this kind as obtained with rat serum are indicated in Fig. 1.

Extensive investigations with other inhibitors of this type have failed to reveal any enzymes other than the cholinesterases that are inhibited by very low concentrations of these inhibitors. Moreover, a comparison of the experimental results (*1*) with the theoretical predictions for a reversible competitive inhibitor (*2*) shows directly that Nu-683 must be acting as a selective inhibitor of the pseudo cholinesterase in the serum preparation (Fig. 2).

There is no similar theoretical criterion by which the selectivity of an irreversible inhibitor can be established. However, it was observed that the results with the irreversible inhibitor diisopropyl fluorophosphonate (DFP) frequently give a higher value for the apparent cholinesterase concentration than that obtained by the above technique with Nu-683. Thus it appears that the results obtained with DFP must be at fault for some reason.

One source of error was revealed by experiments

[1] This work was carried out under the guidance of B. Mendel and with the technical assistance of M. de Jonge.

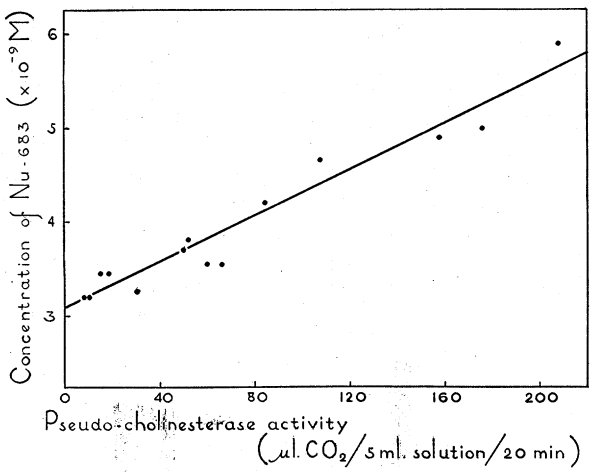

FIG. 1. Determination of the molar concentration of pseudo cholinesterase in rat serum by the use of Nu-683. The experimental points should fall on a straight line of the equation $I_{50} = K_I + 0.5\ E$ (*1, 2*). From the graph, the Michaelis constant $K_I = 3.1 \times 10^{-9} M$ Nu-683, the enzyme concentration $E = 2.45 \times 10^{-9} M$ pseudo cholinesterase in a solution which gives an activity of 100 µl CO_2/5 ml/20 min toward benzoylcholine.

on the inhibition of the pseudo cholinesterase of rat serum by DFP. As in the previous investigation (*1*), the relative enzyme concentration was expressed on the basis of esterase activity, the standard unit of activity being 1 µl CO_2/ml solution/min, with a pH of 7.4, a temperature of 37.5° C, and a 0.025 *M* bicarbonate medium. The pseudo-cholinesterase concentration in rat serum, as determined by the use of Nu-683, was 2.5×10^{-9} *M* in a solution which gives 1 unit of activity toward benzoylcholine. The results with DFP indicated a value that was 250 times larger (Table 1).

It is known that DFP is a moderately potent inhibitor of hydrolytic enzymes other than the cholinesterases (*3–5*), and it had been reported previously that the aliesterases of rat serum show an abnormally high sensitivity to DFP (*6*). Thus it was possible that the presence of the aliesterases in normal rat

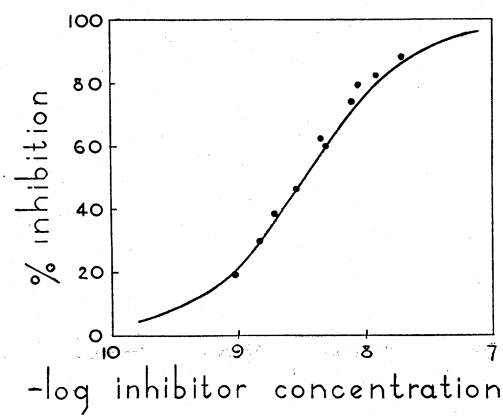

FIG. 2. Relation between the percentage inhibition of pseudo-cholinesterase activity and the logarithm of the concentration of Nu-683. The theoretical line is calculated from the theoretical equations of Goldstein (*2*) for a reversible competitive inhibitor which combines with the enzyme-active center on an equimolar basis. $K_I = 3.1 \times 10^{-9} M$, $E = 1.2 \times 10^{-9} M$.

serum interfered with the reaction between DFP and the pseudo cholinesterase.

In the previous investigation (*6*), ethyl chloroacetate had been used as substrate for the aliesterases, since it is hydrolyzed much more rapidly than the usual substrate, tributyrin. The results with tributyrin, however, can be more easily reproduced than those with ethyl chloroacetate, and the same aliesterase seems to be responsible for the hydrolysis of both esters by rat serum. Thus a 0.2% tributyrin emulsion was used as substrate for the aliesterases in the present investigation.

TABLE 1

Inhibitor	Treatment of serum	Intercept on I_{50} axis	Apparent enzyme concentration in a solution which gives 1 µl CO_2/ml/min toward benzoyl choline
Nu-683	Normal	$3.1 \times 10^{-9} M$	$2.45 \times 10^{-9} M$ pseudo cholinesterase
DFP	"	0	$6.5 \times 10^{-7} M$ pseudo cholinesterase
		0	$4.6 \times 10^{-7} M$ aliesterase
DFP	Heated	$< 0.5 \times 10^{-9} M$	$1.4 \times 10^{-8} M$ pseudo cholinesterase
DFP	TOCP*	$< 0.5 \times 10^{-9} M$	$1.7 \times 10^{-8} M$ pseudo cholinesterase

* TOCP = tri-*o*-cresyl phosphate.

The I_{50} values obtained with DFP indicated an apparent enzyme concentration of 6.0×10^{-8} *M* aliesterase in a solution of normal rat serum which gives 1 unit of activity toward tributyrin. With the particular sample of pooled female rat serum used, the aliesterase activity toward tributyrin was 1390 µl CO_2/ml serum/20 min, as compared with the pseudo-cholinesterase activity of 180 µl CO_2/ml/20 min toward benzoylcholine. Thus a concentration of 4.6×10^{-7} *M* DFP should inhibit the aliesterase in a solution giving 1 unit of activity toward benzoylcholine, whereas the pseudo cholinesterase should be inhibited by 6.5×10^{-7} *M* DFP (Table 1). Apparently the DFP combines preferentially with the aliesterases of rat serum, and the pseudo cholinesterase is inhibited only by the surplus of DFP remaining after the aliesterase has been completely inhibited.

To test this hypothesis, the aliesterase was selectively destroyed in two ways: first by heating the native serum at 53° C for 60 min; and, second, by the intramuscular injection of 10 mg tri-*o*-cresyl phosphate/100 g body weight into female rats (*6, 7*). In both cases the aliesterase activity toward tributyrin was reduced to 1–2% of the former value, whereas most of the pseudo-cholinesterase activity remained unimpaired. And in both cases the elimination of the aliesterase from the serum solution resulted in a forty- to fiftyfold increase in the sensitivity of the pseudo cholinesterase toward DFP (Table 1).

Thus it can be concluded that the high concentration of DFP necessary to inhibit the pseudo cholinesterase in normal rat serum is due, in part at least, to the fact that the DFP combines preferentially with other proteins in the serum—namely, the aliesterase. Although the aliesterases of some other types of serum (e.g., of human serum) are not sensitive to inhibition by low concentrations of DFP (*6, 8*), it is still possible that other enzymes or protein groups might interfere with the reaction between DFP and pseudo cholinesterase in the same way. Inhibitors such as eserine, prostigmine, and analogs, on the other hand, appear to be relatively specific for the cholinesterases. It seems probable that the use of DFP may enable the determination of cholinesterase concentration in highly purified preparations of the cholinesterase (*9*), but the results obtained with crude enzyme preparations cannot always be relied upon, since the presence of other hydrolytic enzymes may protect the cholinesterases against inhibition by DFP.

References

1. MYERS, D. K. *Biochem. J.* (in press).
2. GOLDSTEIN, A. *J. Gen. Physiol.*, **27**, 529 (1944).
3. WEBB, E. C. *Biochem. J.*, **42**, 96 (1948).
4. JANSEN, E. F., NUTTING, M. D. F., and BALLS, A. K. *J. Biol. Chem.*, **175**, 975 (1948).
5. *Ibid.*, **179**, 189, 201 (1949).
6. MYERS, D. K., and MENDEL, B. *Proc. Soc. Exptl. Biol. Med.*, **71**, 357 (1949).
7. MENDEL, B., and MORTIMER, E. Rept. to Natl. Research Council of Canada (Oct. 1944).
8. ADAMS, D. H., and WHITTAKER, V. P. *Biochem. J.*, **44**, 62 (1949).
9. MICHEL, H. O., and KROP, S. *J. Biol. Chem.*, **190**, 119 (1951).

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The Hall Effect and Electrical Resistivity of Tellurium[1]

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At low and high temperatures, tellurium is a P-type semiconductor, but in samples of adequate purity there is a range of temperatures within which the sign of the Hall coefficient is negative. This anomalous behavior, which has been noted by several observers (*1, 2*), has been studied in very pure tellurium prepared by multiple fractional distillation in an atmosphere of helium.

Fig. 1 shows the behavior of the Hall coefficient R, for samples of varying degrees of purity. Curve A is from a sample of c. p. grade tellurium containing an extrinsic carrier concentration of about 10^{18} carriers/cm^3. Curve D is for a sample containing about 10^{15} carriers/cm^3. The remaining curves are for samples of intermediate degrees of purity.

In all samples containing fewer than about 10^{17} carriers/cm^3, R is negative within a certain range of temperature. At low temperature R is always positive, changing to negative at a temperature which depends upon the extrinsic carrier concentration. R remains negative between this point and the temperature 230° C near which the sign becomes positive again and remains so to the melting point. The upper reversal temperature is fixed at approximately 230° C, but the lower reversal temperature is related to the carrier concentration by the empirical relationship

$$\ln R' = A + b/T_r,$$

where R' is the value of the Hall coefficient in the exhaustion range, T_r is the temperature of reversal, and a and b are constants.

In samples cut from single crystals of tellurium, neither the value nor sign of R depends upon the orientation of the sample with respect to the magnetic field or crystallographic axes. Measurements of the Hall effect made with an a-c method show that the anomalous behavior is not due to the Ettingshausen effect. This conclusion has also been verified by direct measurement of the thermoelectric power and Ettingshausen coefficient.

Fig. 2 shows the resistivity as a function of temperature for a number of polycrystalline samples. The intrinsic resistivity at 27° C is approximately 0.5 ohm-cm, but an exact value cannot be given because

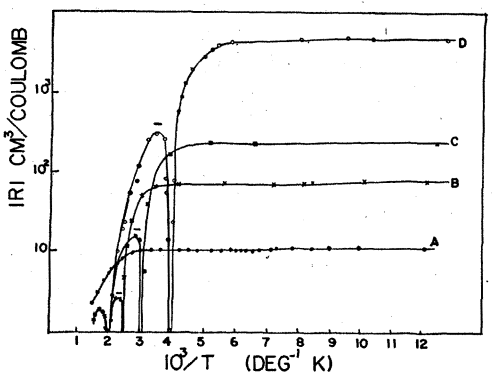

FIG. 1. The Hall effect in tellurium. The logarithm of the Hall constant R is plotted against $1/T$.

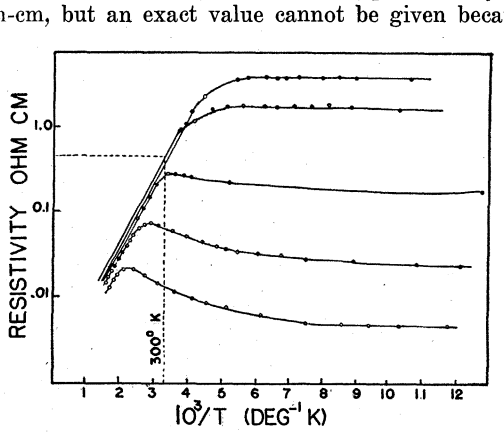

FIG. 2. The resistivity of tellurium measured in polycrystalline samples. Logarithm of the resistivity in ohm-cm plotted against $1/T$.

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[3] Work carried out under contract with the Signal Corps.