manifest after the cultures were subjected to a "physiological shock" brought about by an abrupt change from one medium to another.

Because it was late in the season, it was not possible to inject some of the granules into larvae to observe whether symptoms of a typical polyhedral disease developed. Electron microscope studies of the granules are now in progress (*10*).

THOMAS D. C. GRACE*
Rockefeller Institute for Medical Research, New York, New York

References and Notes

1. R. C. Wallis, *J. Econ. Entomol.* 50, 580 (1957).
2. K. Yamafuji and F. Yoshihara, *Enzymologia* 15, 14 (1951).
3. K. Yamafuji and H. Omura, *Enzymologia* 14, 120 (1950).
4. W. Trager, *J. Exptl. Zool.* 71, 489 (1935).
5. This work was supported in part by a research grant (No. E-1537) from the National Institutes of Health, U.S. Public Health Service.
6. H. J. Eagle, *J. Exptl. Med.* 102, 595 (1955).
7. J. W. Chapman and R. W. Glaser, *J. Econ. Entomol.* 8, 140 (1915).
8. S. S. Wyatt, *J. Gen. Physiol.* 39, 841 (1956).
9. T. D. C. Grace, *Australian J. Biol. Sci.*, in press.
10. A paper describing these studies, as well as observations on the development of the disease in larvae, is in preparation.

* On leave of absence from the Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Australia, February 1957–February 1959.

10 March 1958

Atomic Bomb Effect: Variation of Radiocarbon in Plants, Shells, and Snails in the Past 4 Years

The measurements reported in this paper were primarily designed for a study of the activity of recent organic and inorganic material in various environments. They also gave evidence, however, of a fast increase of the activity of C^{14} in the atmosphere due to atom bombs.

The first group of samples consisted of mussels from the Dutch Waddenzee (*Mytilus edulus*). They are indicated by *Ms* (shells) and *Mf* (Flesh). The second group consisted of land snails (*Helix pomatia*) from a snail farm in Valkenburg (province of Limburg). They are indicated by *W*. The third group consisted of fresh water molluscs (*Dreissenia polymorpha* Pallas) and an alga (*Tolypellopsis stelligera* (Bauer) Migula, family Characeae), collected by G. P. H. van Heusden in the lakes at Loenen (province of Utrecht). These samples are indicated by *Ls* and *Lf* for the dreissenia and by *Lp* for the plant. The lake water contained, on the average, 104 mg of HCO_3^{--} per liter and about 1 mg free CO_2 per liter. The recent calibration sample (*P*) consisted of peanut shells bought in March 1955. These shells probably grew in the summer of 1954. Because of the fast rise in the concentration of C^{14} in the past few years, it is also of importance to give the dates of collection of the other samples; *W*53, *M*, and *L* were collected in the second half of November, 1953; *W*56 on 15 Nov. 1956; and *W*57 on 18 June 1957. The snails collected in November were in a hibernating state.

The activities shown in Table 1 are given by their difference δ from the standard *P* in permillage of the activity of the standard. Each sample has been measured at least twice. The statistical error was between 0.2 and 0.3 percent. This is also the error in δ since the standard *P* has been measured several times.

As a check on fractionation during growth, and so forth, the concentration of C^{13} has been measured (*1*). The results are given in Table 1 by the deviation from the standard *P*. Though some of the measurements were certainly accurate to better than 0.1 percent, sometimes irregularities occurred. Since all measurements were duplicated at least once, the error in δ C^{13} is estimated to be less than 0.1 percent.

Recent increase of concentration of radiocarbon. In looking at the activity *Wf* of the flesh of *Helix pomatia*, which is in fairly fast exchange with the plants the animal lives on, a remarkable increase by 4.3 percent is observed between November 1953 and June 1957. This increase can be due only to production of C^{14} by atomic bombs. A similar effect has been observed in New Zealand (*2*) by a study of CO_2 samples immediately taken from the atmosphere. The increase of the activity of the shells (*Ws*) is only about 1 percent; obviously the carbonate in the shell does not exchange with the environment; the greater part of the shell was deposited in the period of lower C^{14} concentration, but this fraction is difficult to estimate. The owner of the snail farm claims that at least some of the snails may have been up to 10 years old, but a somewhat lower limit is more probable. The lower activity of *Ws*56 as compared with *Ws*53 may be due to a higher age of these shells; it is not possible to check this, however. Furthermore, the activity of *Ws*53 is also too low. These shells contain 1.6 percent more C^{13} than the flesh does. Thus they should contain 3.2 percent more C^{14} (see also below). This is not true at all for the more recent shells where the fast increase of atomic bombs has produced a fast increase of activity in the flesh. The flesh is even more active than the shell. But already by 1953 the flesh was only 1 percent less active, instead of 3.2 percent. This discrepancy has puzzled us for a long time; it probably means that the bomb had produced an increase of about 2 percent by the end of 1953. This is being checked by collection of more appropriate samples. Since the flesh of *Helix pomatia* has the same C^{13} content as the peanut shells, the two should have the same C^{14} content. The difference is probably due to the fast increase of C^{14} in the atmosphere between the two dates of collection, though it is not completely certain that geographic differences have not played a role.

The discrepancy between the enrichment of C^{14} and C^{13} in the shell does not occur for the mussels and the dreissenia. For the mussels this will be mainly due to the fact that the increase of C^{14} concentration in the ocean is much slower. New samples were collected in November 1957 from the same locality in order to obtain some information, which is very important for discussions on exchange between the ocean and atmosphere. The activity proved to be only 0.5 ± 0.3 percent above the activity in 1953. The dreissenia shells were at most 1 year old, and this may explain why no discrepancy occurred.

Isotopic fractionation. Organic and inorganic carbon in the same environment can have different isotopic composition by fractionation in chemical and physical processes. Generally the fractionation is small; then the enrichment of C^{14} should be twice the enrichment of C^{13}. It has been mentioned already above that this does not hold for *Helix pomatia*. The relation is nicely confirmed by the measurements on the three samples from the *Loenensche plassen* (*Ls*, *Lf*, and *Lp*) and the same is true for the samples *Ms* and *Mf*. It is of biochemical interest to note that the fractionation between shell and flesh is not the same for the three animals.

Difference of environment. It is obvious that the *Loenensche plassen* have a low C^{14} content; this had been expected because of the transport of old

Table 1. Summary of results. The notations for the samples are given in the text.

Sample No.	δ C^{14} (per mil)	δ C^{13} (per mil)
P	*0*	*0*
*Ws*53	−11	+16
*Wf*53	−21	0
*Ws*56	−17	*
*Wf*56	+18	*
*Ws*57	−2	*
*Wf*57	+22	*
*Ms*53	−13	+19
*Mf*53	−50	+4
*Ls*53	−44	~+25
*Lf*53	−94	~0
*Lp*53	−82	~+8

* The various batches of *Helix pomatia* (*W*) all gave the same concentration of C^{13}.

carbon with upwelling water into these lakes. The activity of the upwelling water alone is probably about 20 percent below the recent activity (*3*). The activity actually found is about 8 percent below normal; it is not possible to apply a correction for fractionation, for there is probably also no equilibrium for the C^{13} content of the lakes and the atmosphere.

The difference in the C^{14} concentration of the atmosphere and the ocean is of considerable interest. The present measurements may add some information to the data discussed previously in the literature (*5*). The new data cannot be fitted into the picture, however, before an international C^{14} standard is available. Such a standard will permit the expression of all activities relative to the same standard.

H. DE VRIES
Natuurkundig Laboratorium, Groningen, Netherlands

References and Notes

1. I acknowledge the measurement of the C^{13}/C^{12} ratio to Dr. Ir. H. Boerboom and Mr. F. Monterie, Laboratorium voor Massaspectrografie, Amsterdam. For technical details see: *Appl. Sci. Research* B5, 388 (1957).
2. T. A. Rafter and G. J. Fergusson, *Science* 126, 557 (1957).
3. K. O. Münnich, *Naturwissenschaften* 44, 32 (1957).
4. H. Craig, *Tellus* 9, 1 (1957).
5. R. Revelle and H. E. Suess, *ibid.* 9, 18 (1957).

11 February 1958

Some Kinetic Parameters of Liver Glucose-6-Phosphatase in Normal and Diabetic Rats

Several laboratories have recently reported an increase in the activity of liver glucose-6-phosphatase in the diabetic animal (*1–4*) and in animals subjected to increased levels of adrenal cortical hormones (*2, 3, 5*). Insulin appeared to reverse the effect in the diabetic animal within 12 to 48 hours (*1, 2*). Ashmore *et al.* (*1*) further observed that mixtures of normal and diabetic rat liver homogenates possessed additive activities. This fact suggested that the difference was not due to the presence of an inhibitor in normal liver or an activator in diabetic liver, although the possibility of limited amounts of a tightly bound activator or inhibitor is not entirely eliminated. We have undertaken an investigation of the comparative properties of normal and diabetic rat liver glucose-6-phosphatase with the view that the results might bear on the problem of the biochemical mechanism whereby the hormonal effects are mediated (*6*).

In general, there appear to be two underlying enzymic mechanisms which could account for the elevated glucose-6-phosphatase activity in the diabetic animal. Either more of the normal enzyme may be present, or the amount of enzyme present may be unchanged but its properties altered in the direction of an increase in catalytic potency (or a combination of both). The latter implies some structural change in the enzyme which would presumably be manifested in one or more measurable properties. In this connection, the very obvious possibility of an increase in the rate constant for the decomposition of the enzyme-substrate complex into products (k_3) must await extensive purification of the enzyme before it can be tested. However, several kinetic parameters, such as the Michaelis constant (K_s), the equilibrium constant for inhibitor binding (K_i), and the enthalpy of activation of the reaction (ΔH^*) can be measured even in a crude system (bearing in mind the possibility that other substances present in the enzyme preparation might affect the values of these parameters). In all of the experiments reported here, conditions were employed in which it had previously been established that the amount of product liberated was proportional to time, the velocity was proportional to enzyme concentration, and the stoichiometry was that expected.

In Fig. 1 are presented the entire series of determinations of apparent Michaelis constants (K_s) and maximum velocities at infinite substrate concentration (V_m) of the liver glucose-6-phosphatase of normal rats of the Carworth strain and their diabetic litter mates (*7*). Several groups of animals, and both males and females, are included in the series. The diabetic animals had been injected subcutaneously with 20 mg of alloxan monohydrate per 100 g at least 3 weeks previous to sacrifice; they had blood sugar concentrations of at least 400 mg/100 ml. From Fig. 1 it can be seen that the average K_s of the diabetic animals was almost twice as great as that of the normal animals, and that there was very little overlap in the values. In addition, a marked linear correlation between the K_s and V_m values for individual animals is apparent ($r = 0.87$). An alteration in k_3 produces a relationship of this kind (*8*).

The ΔH^* values of the reactions catalyzed by the normal and diabetic enzymes over the range 25° to 40°C were 12,480 cal and 16,700 cal, respectively, at a substrate concentration of $0.01M$. The thermal instability of glucose-6-phosphatase at 37°C and *p*H 5.0, which has been reported for the normal enzyme (*9*), was also found in the case of the diabetic enzyme.

Studies of the kinetics of glucose inhibition revealed that the inhibition was of a noncompetitive nature (Fig. 2). The possibility that this effect was the result of an exchange between the substrate and the inhibitor was tested and excluded. The K_i of glucose binding was 0.11 to $0.15M$ (av. $0.12M$) for the enzyme from four normal animals, and 0.11 to $0.18M$ (av. $0.13M$) for the enzyme from seven diabetic animals (*10*).

The results reported here make it clear that while there is an increased *capacity* of the diabetic liver to hydrolyze glucose-6-phosphate, as expressed by the activity per milligram of tissue at high substrate concentration (V_m), the

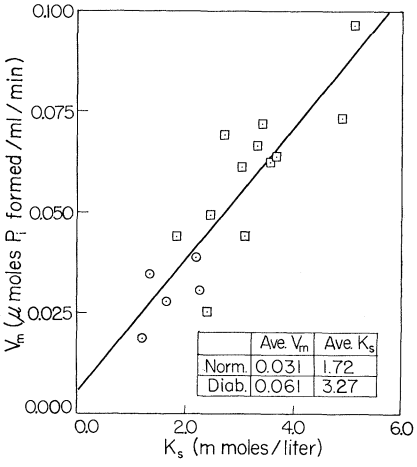

Fig. 1. Kinetic parameters of normal and diabetic liver glucose-6-phosphatase. The normal values are shown by circles, the diabetic by squares. Each value was calculated by the method of least squares from the initial velocities at four to six initial substrate concentrations over the range 1 to 30 μmole/ml of K glucose-6-phosphate (see Fig. 2). In addition, each milliliter of reaction mixture contained 0.3 ml of $0.25M$ sodium cacodylate (*p*H 6.4) and 2.5 mg of liver homogenate. Incubation was for 10 min at 30°C with shaking. Inorganic phosphate (P_i) was determined by the Fiske-Subbarow method. The slope of the line of best fit through the points is 0.017 ± 0.002 min^{-1}.

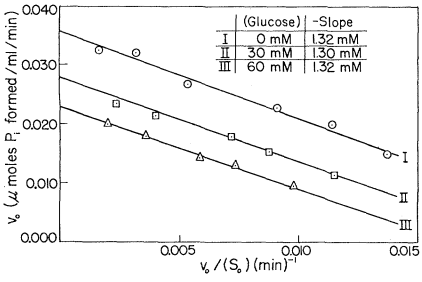

Fig. 2. Glucose inhibition. Incubation conditions were the same as those shown for Fig. 1, except that glucose was added to initial concentration indicated. Initial velocity is plotted against initial velocity divided by initial substrate concentration. Slopes were calculated by the method of least squares.