ples was relatively high, a method was developed to extract and collect the carbon dioxide gas component from ice for radiocarbon dating. The technique was to melt the ice under vacuum and boil the meltwater, all the while removing the gas through a solution of barium hydroxide (Fig. 1).

This technique was tested during April 1957 on Storbreen, the Norwegian glacier examined previously. About 5000 kg of ice (approximately 50 "runs" of the extraction apparatus) were required to obtain enough C^{14} for a reasonably accurate count. Three weeks were required for mining and transporting the ice and for extracting the gas.

The resulting barium carbonate was taken to the Physical Laboratory at the University of Groningen for measurement of the age of the carbon dioxide (*9*). The age obtained for terminal Storbreen ice was 710 ± 120 years. Since only 0.3 g of carbon was available, the sample was counted twice during 5 days (*10*). The age is in excellent agreement with age estimates made by the Norwegian Polar Institute. It appears that this is a suitable technique for the dating of glacier ice.

In conclusion, glacier ice contains gases in sufficient quantity for accurate analysis. Present evidence indicates that, although changes may occur in the gas enclosed in temperate glaciers, the gas trapped in high-polar ice is well preserved. The composition of the enclosed gas yields information on the amount of meltwater that was present in the firnification process, and hence on the climate at the time the ice was formed.

Information on the composition of the atmosphere at the time of ice formation is also obtained. An investigation of gas in icebergs (*11*) showed one of the icebergs to have an absolutely uniform gas composition with respect to oxygen, indicating unchanged atmosphere. It would seem that, through further investigation in Greenland with the techniques now available, it may be possible to locate, analyze, and date ancient atmosphere in its original state.

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References and Notes

1. P. F. Scholander *et al.*, *J. Cellular Comp. Physiol. Supp.* 42, 1 (1953).
2. L. K. Coachman *et al.*, "Gas enclosures in Greenland ice," in preparation.
3. L. K. Coachman *et al.*, *Tellus* 8, 4 (1956); L. K. Coachman *et al.*, "Gas loss from a temperate Glacier," in preparation. These studies were aided by a contract between the Office of Naval Research, Department of the Navy, and the Arctic Institute of North America.
4. E. Gorham, *Tellus*, in press.
5. E. Hemmingsen *et al.*, "Diffusion of gases through ice," in preparation.
6. R. F. Flint, *Glacial Geology and the Pleistocene Epoch* (Wiley, New York, 1947); H. W. Ahlmann, *Glacier Variations and Climatic Fluctuations* (American Geographical Society, New York, 1953).
7. R. Revelle and H. E. Suess, *Tellus* 9, No. 1, 18 (1957).
8. S. Arrhenius, *Lehrbuch der kosmischen Physik* (Hirzel, Leipzig, 1903), vol. 2; T. C. Chamberlin, *J. Geol.* 7, 575 (1899).
9. H. de Vries *et al.*, *Physica* 19, 987 (1953); H. de Vries, *Appl. Sci. Research* 5B (1955).
10. Station numbers GRO-750 and -758.
11. P. F. Scholander *et al.*, *Science* 123, 3186 (1956).

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Sensitivity to Oxygen During Postembryonic Development of the Wasp Habrobracon

Work on the effects of oxygen gas upon *Habrobracon juglandis* (Ashmead) has shown that pupae are injured by exposure to oxygen whereas larvae appear to be unaffected (*1*). Pupae exposed to 30 lb of oxygen for 1 minute showed an immediate and marked decrease in oxygen uptake as well as arrested development. Since pupae and larvae differ considerably in their sensitivity to oxygen gas, it seemed pertinent to determine at what stage the change from oxygen-resistance to oxygen-sensitivity occurred. Since injury from exposure to oxygen in *Habrobracon* can be established by measuring the oxygen consumption or by determining the incidence of embryos that are able to develop to the adult stage (*1, 2*), we decided to study the stage-sensitivity to oxygen gas, using these criteria. In the present report the effects of exposure to oxygen upon the oxygen consumption at various stages of postembryonic development is reported (*3*).

The wasp *Habrobracon* is parasitic upon the larvae of the Mediterranean flour moth, *Ephestia kuhniella* (Zeller). Female wasps were placed in dishes containing *Ephestia* larvae and were permitted to lay eggs upon them. After a 2-hour period the wasps were transferred to other dishes containing fresh *Ephestia* larvae. Cultures were allowed to develop to the desired stage for treatment with oxygen (Table 1). The life-cycle from egg to imago takes about 8½ days at 31°C. The cultures used consisted entirely of haploid males and were obtained parthenogenetically from unmated mothers.

Individuals of known age and stage of development were placed in plastic chambers of approximately 100 cm^3 volume. Oxygen gas was flushed through the chamber for 1 minute in order to remove the air and was then applied for an additional minute at 30 lb pressure. The cultures were then removed from the chambers, flushed with air, placed in a flask, and measured for oxygen consumption about 1 hour after treatment, by means of the Warburg apparatus. Determinations were made on groups of 25 wasps for a 3-hour period. Measurement of oxygen consumption for an additional 3 hours showed no change from the first 3 hours. The oxygen-treated groups were paired with air-exposed control groups so that a measure of the degree of injury could be obtained by determining the ratio of oxygen consumed by experimentals to that consumed by the controls (see Table 1, column 5). Other individuals in the cultures were examined in order to determine whether the oxygen-treated embryos could develop to the adult stage.

The oxygen consumption for *Habrobracon* exposed to air or to 30 lbs of oxygen at certain stages of postembryonic development is presented in Table 1. Each value and standard error is based upon from three to six experiments. Comparison of the "oxygen consumed" values for experimentals and controls shows that for some stages of development there is no significant difference while for others the difference is large. Comparison of the values for magnitude of oxygen consumption, with age, for the controls shows the U-shaped curve that has been reported for many insects. Cultures that are oxygen-treated as larvae in cocoons or as prepupae show no decrease in oxygen consumption from that of their control groups. Furthermore, cultures at these stages were able to develop normally and to emerge as adults. Cultures consisting of a mixture of prepupae and white pupae show a significant difference in oxygen consumption between controls and experimentals. This difference is more pronounced for white pupae (120 hours and 144 hours old) and for pigmented pupae (168 hours old). The oldest pigmented pupae (192 hours) show a decrease in sensitivity (Table 1).

The decrease in oxygen consumption reported here for the oxygen-treated pupae is a permanent decrease. Experiments reported elsewhere (*2*) have shown that there is no subsequent increase in oxygen consumption for these pupae, even after 24 and 48 hours. The effect reported here is irreversible.

Wasps in these stages of development show not only a decrease in oxygen consumption after oxygen treatment but arrested development as well, so that most of these insects do not reach the adult stage. Exposure of white pupae to 30 lb of oxygen for 1 minute will cause over 95 percent of these wasps to remain as white pupae (*2*). This procedure is now

Table 1. Oxygen consumption of *Habrobracon* exposed to 30 lb of oxygen.

Age (hr)	Stage of development	Oxygen consumed/μl hr 25 wasps		Oxygen consumed (percentage of amt consumed by controls)
		Air-exposed controls	Oxygen-treated	
80	Larva in cocoon	98 ± 6	92 ± 2	94
96	Larva in cocoon	98 ± 5	97 ± 5	99
112	Prepupa	55 ± 3	56 ± 2	102
116	Prepupa and white pupa	58 ± 2	39 ± 4	67
120	White pupa	53 ± 3	24 ± 7	45
144	White pupa	42 ± 4	4 ± 1	10
168	Pigmented pupa	37 ± 1	10 ± 1	27
192	Pigmented pupa	62 ± 6	47 ± 4	76

used routinely in our laboratory in order to arrest the development of pupae so that they can be used for various physiological studies. Although both a decrease in oxygen consumption and arrested development result from exposure of pupae to 30 lb of oxygen, it is not clear at present in what way, if at all, these two types of injuries are related. It appears from these data that injury from exposure to oxygen occurs with the onset of the white pupal stage and that there is a greater oxygen-sensitivity in older white pupae and in younger pigmented pupae than in wasps in the other stages of development.

These studies show that individuals of *Habrobracon* in certain stages of development are injured by oxygen while those in other stages apparently are unaffected. The reason for this stage-influenced sensitivity is not known and is a point of interest in continuing investigation.

It seems clear, however, that this difference in sensitivity is not due simply to a difference in the rate of metabolism. Although larvae are more active and also more oxygen-resistant than pupae, pupae whose rate of metabolism has been lowered by exposure to lower temperature (10°C) are more oxygen-resistant than pupae whose rate of metabolism is higher due to exposure to temperature of 26°C before treatment with oxygen (*2*). It is commonly known that organisms are more sensitive to oxygen when in a higher metabolic state than when their metabolism has been lowered. Adults of *Drosophila azteca* whose metabolic rate had been raised by elevation in temperature were more sensitive to oxygen poisoning than flies of lower metabolism (*4*). In rats, the lowering of metabolism by removal of the adrenal cortex reduces oxygen sensitivity, while the injection of adrenal cortex extract into adrenalectomized rats increases their oxygen-sensitivity (*5*).

If some basic cellular process is involved, it seems probable that the difference in sensitivity between larvae and pupae would be of a quantitative nature but that both would be affected. Since pupae are injured but larvae or prepupae are not, it appears that some oxygen-sensitizing process occurs in the pupal stage but not in the larval or prepupal stages. A comparison of metabolism at these various stages of development is required to determine the processes involved.

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References and Notes

1. A. M. Clark and E. B. Herr, Jr., *Biol. Bull.* 107, 329 (1954).
2. A. M. Clark and M. J. Papa, *ibid.* 114, 180 (1958).
3. This research was carried out under A.E.C. Contract AT(30-1)-1752 between the University of Delaware and U.S. Atomic Energy Commission.
4. C. M. Williams and H. K. Beecher, *Am. J. Physiol.* 140, 566 (1944).
5. R. Gerschman *et al.*, *ibid.* 178, 346 (1954).

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Double Monochromation in Ultraviolet Microspectrophotometry

Ultraviolet microspectrophotometers include, besides a light source and a detector (photocell or multiplier), a monochromator and a suitable ultraviolet microscope. To avoid excessive irradiation of the object, the monochromator is usually placed between the light source and the microscope.

Several difficulties had to be met in constructing a workable microspectrophotometer. Chromaticity of the optical system, such as is encountered in Zeiss monochromats, has been overcome by the use of catoptric or catadioptric objectives, as in the systems designed by the American Optical Company and by Bausch & Lomb. Fluctuations of the light source that produce absorptions or transmissions not related to the object have been minimized, either by stabilizing the sources or by introducing double-beam systems. Nonlinearity of the detectors has been corrected by using null methods—for example, by inserting a rotating variable sector into the light beam passing through the microscope. It is the reading of the sector aperture which gives the transmissivity in this case, and the detectors monitor constant intensities only.

And yet, even arrangements employing all these features work well only if certain additional conditions are fulfilled. The apparatus ought to be used in a spectral region where the output of the lamp is high and the detector exhibits a high sensitivity to the incident signal. These restrictions are necessary because of the stray light present when a single monochromator is used. At the exit slit, not only light of the "nominal" wavelength but also spurious scattered and reflected light, embracing the entire spectrum, is present. Such over-all random light may amount to several percent of the total radiation.

The random light results in a significant number of false readings in spectrophotometry and microspectrophotometry, especially when transmission measurements are made in a spectral region where there is little emission from the light source or when the response of the detector is weak, or when both these conditions exist. (It is immaterial whether this decrease in response is due to the characteristic of the sensitive surface of the detector or to a strong absorption in the optical train, including the envelope of the detector.) The situation is still further complicated if the specimen strongly absorbs at the nominal wavelength setting of the monochromator. Coincidence of some or of all of the factors listed necessarily results in serious errors when stray light, at or near the peak sensitivity of the detector, is not absorbed by the optical system, including the specimen and cell envelope. Under such circumstances Halban and Eisenbrand (*1*) recorded errors of up to 75 percent.

Hogness and his coworkers (*2*), Deck (*3*), Zscheile (*4*), Gibson (*5*), and others have established the conditions for precise spectrophotometry. Caspersson (*6*), Sinsheimer (*7*), and Walker and others (*8*) have established those of microspectrophotometry (see also Harrison *et al.*, *9*, and Kortüm, *10*). It is now generally acknowledged that double monochromation is essential for exact transmission measurements. If initially monochromatized light, containing 2 percent of stray light, is fed into a second,