sind weder vor noch nach dem Stimmbruch nachzuweisen. War bei einer Taube der Untersuchungszeitraum, in dem Kot gesammelt wurde, auf wenige Tage vor dem Stimmbruch beschränkt, so ergaben die Messungen eine im Mittel höhere tägliche Ketosteroidausscheidung im Gegensatz zu solchen Tauben, bei denen sich Untersuchungszeitraum auf 2–3 Wochen vor dem Stimmbruch erstreckte. Deren niedrige Werte ergeben sich aus der Mittelung über anfänglich sehr geringen und später erst ansteigenden höheren Ketosteroidausscheidungen. In der Figur sind die Änderungen der mittleren Frequenzen der Lautäusserungen in der Stimmbruchphase und die Ketosteroidausscheidung derselben Tauben in dieser Phase in Abhängigkeit vom Untersuchungszeitraum dargestellt. Da der Zeitraum, in dem das Untersuchungsmaterial der einzelnen Tauben gesammelt wurde, unterschiedlich war, ergibt sich nach Umrechnung auf 1 kg Körpergewicht und 24 h eine Punkteschar jeweils für die Fraktionen vor und nach dem Stimmbruch.

Vier Tage vor dem Stimmbruch sind die Frequenzen signifikant gegenüber denjenigen 10 Tage vor Stimmbruch um über 400 Hz abgesunken (U-Test von Mann-Whitney, p 0,03, Frequenzwerte sind nicht normal verteilt, wie der Lilliefors-Test von Kolgomorow-Smirnow ergab). Es tritt also eine erste erkennbare Änderung der Frequenzen rund 10 Tage nach einer deutlich erhöhten Steroidausscheidung auf. Auch bei Hausenten und Haushähnen verstreichen ungefähr 10 Tage zwischen einer Erhöhung der Gonadenaktivität und dem Absinken der Frequenzen der Lautäusserungen (ABS, unveröffentlicht). Geschlechtsunterschiede in der Frequenz der Lautäusserungen – die Frequenzen der Lautäusserungen von Weibchen liegen um rund 40 Hz über denjenigen von Männchen – sind erst 24 Tage nach dem Stimmbruch nachweisbar (t-Test nach Student, p 0,01).

Aus dem Anstieg der Werte der Steroidausscheidung mit Annäherung an das Stimmbruchsdatum lässt sich die These ableiten, dass die Ketosteroidausscheidung mit dem Eintritt in die Stimmbruchphase exponentiell anwächst und zum Zeitpunkt des Stimmbruchs ein Maximum durchläuft, um anschliessend wieder auf ein niedrigeres Niveau abzusinken. In der Figur ist diese Hypothese wiedergegeben durch den Verlauf von Gompertzfunktionen [vor Stimmbruch: $y = 1{,}2 + 16{,}5 \exp(-0{,}09 \exp 0{,}2x)$; nach Stimmbruch: $y = 4 + 15{,}2 \exp(-0{,}09 \exp 0{,}2x)$], die den experimentellen Werten angepasst wurden. Ob die angenommene erhöhte Ketosteroidausscheidung bei Eintritt in die Stimmbruchphase den Schluss auf einen gesteigerten Testosteron-Stoffwechsel zulässt, bleibt offen. Dabei ist nämlich zu berücksichtigen, dass RIVAROLA et al.[6] im Plasma adulter Tauben mehr Androstendion und DHEA als Testosteron nachgewiesen haben.

Eine Auftrennung des Ketosteroidgemisches aus den Kot-Harn-Proben von jungen Tauben mit dem oben beschriebenen Dünnschicht-Chromatographiesystem zeigte, dass das Gemisch aus wenigstens 7 verschiedenen Komponenten besteht (Farbreaktion mit Zimmermann's Reagens nach NEHER[7].) Eine Identifizierung der Komponenten brachte bisher keinen Erfolg[8].

Summary. The daily ketosteroid excretions from 12 juvenile pigeons were measured during the phase of the breaking of the voice, i.e. from 5 to 12 weeks of age after hatching. The concentrations of the ketosteroids were determined photometrically with the Zimmermann-reaction after extraction of the collected voidings. During the days before the breaking of the voice, the ketosteroid concentrations are elevated. After the breaking of the voice, they sink to a low level.

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4. März 1974.

[6] M.A. RIVAROLA, CH.A. SNIPES and C.J. MIGEON, Endocrinology *82*, 115 (1968).
[7] R. NEHER, *Steroid chromatography* (Elsevier, Amsterdam 1964).
[8] Berechnungen innerhalb dieser Arbeit wurde mit der Rechenanlage PDP 12 durchgeführt, die die DFG Herrn Prof. Dr. J. SCHWARTZKOPFF zur Verfügung gestellt hat.
[9] Herrn Prof. Dr. G. Niethammer zum Gedächtnis.

PRO EXPERIMENTIS

An Isotope Dilution Method for the Determination of Dissolved Carbon Dioxide

In order to estimate primary production in natural waters by the radio-carbon method[1], it is necessary to know precisely the amount of inorganic carbon compounds present in those waters. The numerous published methods for this determination divide into two main classes[2]; a) those in which the pH buffering capacity of the water are used to calculate the inorganic carbon content. b) those in which the inorganic carbon compounds are liberated from the water, and assayed separately. We shall subsequently use the term dissolved carbon dioxide to encompass all components of the equilibrium, i.e. carbon dioxide, carbonic acid, carbonate, and bicarbonate ions.

Methods in Class [a] are simple to perform, but only give satisfactory results in oligotrophic lakes, the open sea and similar situations, and are not suitable for highly polluted waters or waters of variable salinity. Methods in Class [b] tend to require sophisticated or delicate equipment and are only suitable for research laboratories or large and well equipped vessels.

Radioactive isotope dilution analysis, however, offers an alternative to these methods with their inherent limitations, by removing the need for a quantitative recovery, while at the same time providing an extremely sensitive internal standardization. We have therefore adopted this approach in the development of our method for measuring total dissolved carbon dioxide in aqueous solution, a need which arose during the course of our studies of primary production in some Western Scottish sea lochs[3].

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[2] T. R. MILBURN and L. C. BEADLE, J. exp. Biol. *37*, 449 (1960).
[3] B. J. B. WOOD, P. B. TETT and A. EDWARDS, J. Ecol. *61*, 569 (1973).

We were using the standard carbon-14 technique for estimation of primary production, and decided to develop a method which used this radioisotope as the internal standard. Originally we intended to take filtered water from the production study, distil off the carbon dioxide in a stream of CO_2-free air and collect both the $^{14}CO_2$ and the $^{12}CO_2$ in aqueous alkali. The total mass of carbon dioxide trapped in the alkali (which is essentially $^{12}CO_2$) could be determined by measuring the change in electrical conductivity of the solution, and the activity of the $^{14}CO_2$ determined by liquid scintillation. Knowing the amounts of cold carbon dioxide and of radioactive carbon dioxide present in the alkali, the amount of radioactive carbonate originally added to the water, and assuming no isotopic rate effects in the distillation, it is a simple matter to calculate the amount of carbon dioxide originally present in the water sample. This procedure wold remove the need for quantitative recovery. However, the paper by JOHNSON and MICHALSKI[4] suggested a further simplification, permitting many samples to be handled at once; use of the radio-isotope dilution technique meant that the entire analysis could be completed in about 5 h.

The final form of our method is as follows: Water samples (approximately 315 ml) are taken, inoculated with the radioactive carbonate and incubated according to standard procedures. At the completion of the incubation period, the bottles are weighed to determine the volume of water which they contain. The sample is then filtered through a 0.45 μm membrane filter, employing the usual precautions, and in addition the connection between the Buchner flask and the atmosphere is protected by a soda-lime tube. On completion of filtration, about 300 ml of the filtrate is expeditiously transferred to a 250 ml conical flask containing a magnetic stirring rod. The flask is then stoppered with a tightly fitting rubber bung which has been bored with 2 holes. Through the larger hole a well rinsed latex fingerstall (London Rubber Co. Ltd., wall thickness approximately 0.095 mm, volume 15 ml) has previously been threaded and is sealed with a smaller bung (Figure 1). The smaller hole is closed with a glass plug. The 10.0 ml of absorbant (0.1 *N* NaOH) is quickly introduced to the fingerstall and tle small bung replaced. With a little practice, one can perform this operation without unduly exposing the absorbant to the atmosphere, and any alteration in the initial conductivity of the solution is consequently avoided. Finally the water sample is acidified to pH 3.0 with N-hydrochloric acid, introduced by syringe through the smaller hole. The assemblage is then placed on a 16-place magnetic stirring table (manufactured to our specifications by Durward-Clarkson Limited, Anniesland, Glasgow) and stirred at 200 rpm for 3–4 h at ambient temperature. Under the conditions described, we find that 10–20% of the carbon dioxide in the water sample is transferred to the absorbant.

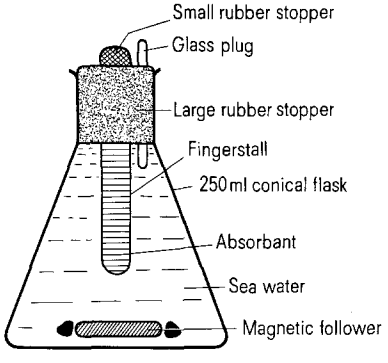

Fig. 1. Assemblage used to secure partial transfer of $^{12}CO_2$ and $^{14}CO_2$ from sea water to dilute alkali.

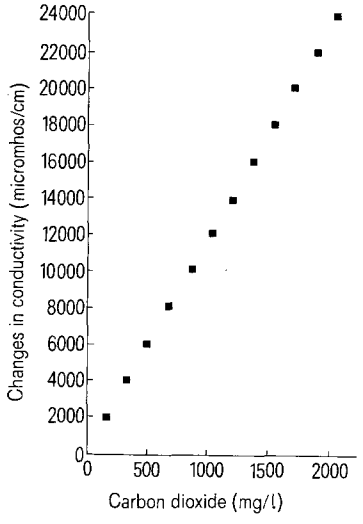

Fig. 2. Relation between the change in conductivity (MicroMhos/cm) and carbon dioxide concentration in the absorbant.

The flasks are then heated to 25 °C by immersion in a water bath for 1 h. Next, the conductivity of the absorbant is measured using a conductivity cell which will slip into the fingerstall; thus this measurement can be made very rapidly. The relationship between conductivity and carbon dioxide concentration is linear in the range 0 to 22 mg carbon dioxide per 10 ml of 0.1 *N* alkali (Figure 2). Finally an aliquot of the absorbant is taken and added to a water-miscible scintillation fluid, and the radioactivity is measured after the mixture has been stored for about 2 h to permit an initial, high, spurious rate of scintillation to disappear. A small increase in counting efficiency ($< 2\%$) occurs with increasing carbon dioxide concentration, and should be corrected for.

Our main source of error was the inexpensive conductivity bridge which we used in these development studies, but even so the experimentally determined precision of the method over the range 50 to 200 mg $CO_2\,l^{-1}$ was $\pm 13\sqrt{n}$ (where n = the number of independent determinations – one in the present case – and the range represents the 95% confidence limits).

The addition of yet another to the array of published analyses for the determination of dissolved carbon dioxide can be justified only if it represents a real advance. We believe that the method which we describe affords a number of advantages: 1. the determination can be carried out easily; 2. the isotope dilution method offers a great sensitivity, which is further enhanced by the concentration of the carbon dioxide from a large volume of water into a small volume of dilute alkali; 3. the measurements of dissolved carbon dioxide concentrations are actually made on the water samples used for the estimates of carbon fixation by the radio-carbon method; 4. the samples can be stored for some time, provided they are protected

[4] M. G. JOHNSON and M. F. P. MICHALSKI, Limnol. Oceanogr. *15*, 481 (1970).

from contamination by atmospheric carbon dioxide and carbon dioxide produced by microbial metabolic activities. Any moderate losses of carbon dioxide will be inconsequential, since both the radio- and stable-isotopes will be lost in equivalent proportions and so will not influence the answer obtained upon analysis; 5. the method is applicable to waters not suited to analysis by conventional field methods.

Riassunto. Si descrive un metodo per determinare il contenuto di anidride carbonica dell'acqua, che prevede il passaggio del CO_2 dall'acqua attraverso una membrana di lattice ad una soluzione alcalina dove la variazione di conduttività e radioattività viene misurata e usata per calcolare il livello di CO_2 del campione d'acqua.

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On Matrix Rank Analysis of Spectra of Multicomponent Mixtures

Several workers have shown that application of the concept of the rank of a matrix to the spectra of multicomponent mixtures can give the number of components in the mixtures. The practical problem of rank determination has been attacked in various ways including evaluation of determinants[1-3], annihilation of rank[4,5], and evaluation of eigenvalues[6]. The rank determined is that consistent with the error of the matrix elements.

Consider a matrix $\boldsymbol{D} = [d_{\lambda i}]$ whose columns consist, for example, of absorption spectra of a multicomponent mixture. Here $d_{\lambda i}$ represents the absorbance at the λth wavelength in the ith spectrum. We assume that each $d_{\lambda i}$ has a variance $var\ (d_{\lambda i})$, and that the $d_{\lambda i}$ are observationally independent. To find the rank of $\boldsymbol{D}$, HUGUS and EL-AWADY[6] recently proposed the determination of the number of nonzero eigenvalues of the real symmetric matrix $\boldsymbol{DD}^T$, where $\boldsymbol{D}^T$ is the transpose of $\boldsymbol{D}$. These authors determined the eigenvalues of $\boldsymbol{DD}^T$ by the Jacobi method. To set up a test for a zero eigenvalue, they considered the propagation of errors in the computation of $\boldsymbol{DD}^T$ and the diagonalization of this matrix. The purpose of this communication is to show that when the elements of $\boldsymbol{D}$ have the same variance it is easier to proceed as follows by considering the singular values of $\boldsymbol{D}$.

A general $m \times n$ matrix of rank k has exactly k positive nonzero singular values[7]. The singular values are real. Corresponding to each singular value μ_p there is a pair of singular vectors $\boldsymbol{u}_p$ and $\boldsymbol{v}_p$. These are eigenvectors of $\boldsymbol{DD}^T$ and $\boldsymbol{D}^T\boldsymbol{D}$ (for a real matrix $\boldsymbol{D}$), respectively, corresponding to an eigenvalue $\lambda_p = (\mu_p^2)$. We have

$$\boldsymbol{D}^T \boldsymbol{u}_p = \mu_p \boldsymbol{v}_p.$$

Multiplying on the left by $\boldsymbol{v}_p^T$ we get

$$\mu_p = \boldsymbol{v}_p^T \boldsymbol{D}^T \boldsymbol{u}_p,$$

since the $\boldsymbol{v}_p$ are orthonormal. If $\boldsymbol{D} = [d_{\lambda i}]$ we have

$$\mu_p = \sum_{\lambda=1}^{m} \sum_{i=1}^{n} u_\lambda\, v_i\, d_{\lambda i}$$

where u_λ and v_i are the λth and ith components of the eigenvectors $\boldsymbol{u}_p$ and $\boldsymbol{v}_p$, respectively. By propagation of errors we have

$$var\ (\mu_p) = \sum_{\lambda=1}^{m} \sum_{i=1}^{n} (u_\lambda\, v_i)^2\ \text{var}\ (d_{\lambda i})\ .$$

It will be noticed that when the $d_{\lambda i}$ have the same variance

$$var\ (\mu_p) = var\ (d_{\lambda i}),$$

since the eigenvectors $\boldsymbol{u}_p$ and $\boldsymbol{v}_p$ are orthonormal, and the problem of determining the number of nonzero singular values of $\boldsymbol{D}$ is greatly simplified. The singular values are computed as the non-negative square roots of the eigenvalues of $\boldsymbol{D}^T\boldsymbol{D}$ or $\boldsymbol{DD}^T$ and compared with the standard deviation of the elements of $\boldsymbol{D}$.

We have tested the method of rank determination given here on an 8×8 matrix of neutral red spectra consisting of 8 rows at different wavelengths and 8 columns at different pH values of the solution as given by WALLACE and KATZ[4]. These authors found the matrix to be definitely of rank 3 and possibly of rank 4 by considering the propagation of errors after reduction steps consisting of Gaussian elimination with complete pivoting. HUGUS and EL-AWADY[6] declared the matrix to be of rank 3 as a result of computing 3 nonzero eigenvalues for the product matrix $\boldsymbol{DD}^T$. The matrix elements each had an error (standard deviation) of 0.003 absorbance unit.

Diagonalization of the product matrix $\boldsymbol{DD}^T$ was carried out by the Jacobi method using an algorithm of RUTIS-

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[7] D. NOBLE, *Applied Linear Algebra* (Prentice-Hall Inc., New Jersey 1969), p. 335.

Computations for 8×8 matrix $\boldsymbol{D}$ of neutral red spectra of WALLACE and KATZ[4]

Eigenvalue of $\boldsymbol{DD}^T$	Singular value of $\boldsymbol{D}$
11.570321	3.401518
1.158019	1.076123
0.096982	0.31142
0.008896	0.094317
0.000068	0.008219
0.00001	0.003115
0[a]	0
0[a]	0

[a] Declared zero after computation as a negative number with small absolute value.