of time. At the end of the study, the amount of carbon dioxide produced is determined by admitting alkali into the vessel (*10*). In these latter methods, the assumption is made that the RQ is constant during the interval of measurement, a condition that is often not the fact (*1, 4*). To obviate these difficulties and to permit a rapid, valid measurement of the RQ, a modification of the standard Warburg flask has been developed for use with a constant-volume respirometer.

The RQ measuring flask (*11*) permits the determination of the carbon dioxide production and the oxygen uptake of a single tissue sample during immediately adjacent intervals, thus eliminating the need for duplicate samples in two separate flasks. The flask (Fig. 1) has one or two hollow stopcock side arms into which folded filter paper and alkali are placed. These side arms replace the usual center well. The hollow stopcock has a large hole (bore) in one side that opens into the central chamber and exposes the absorption surface of the filter paper to the vessel atmosphere. When the stopcocks are in the open positions, the flask measures oxygen uptake in the same way that a standard flask with alkali in the center well does. When the stopcocks are closed, the flask serves as the pair member without alkali in the Warburg direct method (*12*).

The procedure for determining the RQ involves the use of three different time intervals, the first two of which are equal in length. In the first interval, the stopcocks that contain KOH are opened into the chamber, and the change in pressure is proportional to the oxygen uptake; during the second interval, the stopcocks are closed off, and the change in pressure is proportional to the net effect of the carbon dioxide produced and the oxygen uptake. The change in pressure recorded on the manometer during these two intervals, together with the known vessel constants, permits the computation of the RQ (*13*). In the third interval, the stopcock is again opened in order to permit the absorption of the carbon dioxide released during the second and third intervals; this sequence, starting with the first interval, may then be repeated. In principle, this procedure is similar to the one recommended for a flask by Gaffron (*9*).

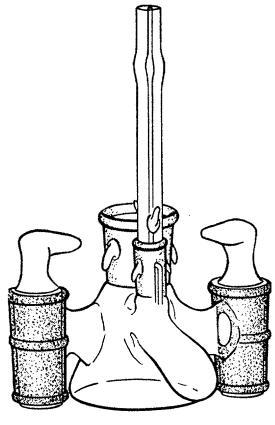

Fig. 1. Manometer flask for measuring respiratory quotients.

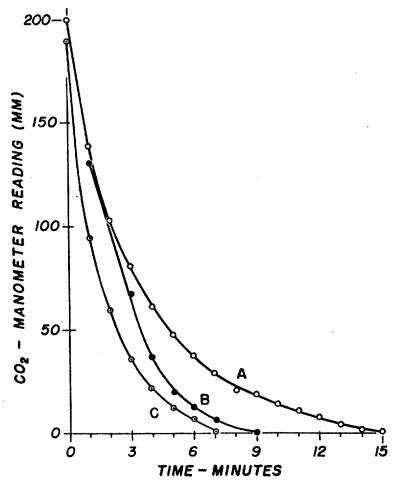

Fig. 2. Effectiveness of RQ flask in absorbing carbon dioxide. (*A*) RQ flask with one alkali-containing stopcock; (*B*) standard flask with center well; (*C*) RQ flask with two alkali-containing stopcocks. The vessels were of 6- to 7-ml volume; similar results were obtained with vessels of 20-ml volume. The main compartment contained 0.5 mg of Na_2CO_3; 0.25 ml of 10-percent oxalic acid was added from the side arm at zero time. Stopcocks contained 0.15 ml of 20-percent KOH and filter paper. The center well contained 0.05 ml of 20-percent KOH and filter paper. Bath temperature was 30°C; Lardy-type Warburg, shaking at 112 oscillations/min.

One recognized limitation of the standard flask is the rate at which the alkali in the center well absorbs carbon dioxide. Figure 2 compares the rate of carbon dioxide absorption in the RQ flasks with that in the standard Warburg flasks. It is apparent that the RQ flask with both stopcocks open is as efficient in carbon dioxide absorption as is the standard Warburg flask with KOH and filter paper in the center well. By using the procedure of Dixon and Elliott (*14*), the time required to absorb a given volume of carbon dioxide may be computed. Thus, the shortest time interval can be determined for which each flask may successfully be used with a given rate of carbon dioxide evolution. In studies conducted upon germinating seeds at this laboratory, the rate of evolution of carbon dioxide and of change in RQ has been well within the absorption capacity of all flasks in use.

A more serious limitation of the standard Warburg flasks, which is in part circumvented by the use of these RQ flasks, is that certain tissues and cells give significantly different respiratory patterns and rates when they are in an atmosphere in which the carbon dioxide tension is reduced to zero (*3*). In the RQ flask the tissue is respiring in the absence of carbon dioxide for only the minimal amount of time required to measure the oxygen uptake; this is closer to the ideal situation than is the case in the usual Warburg direct method.

Other suggested uses of the RQ flask are for the measurement or absorption of metabolically produced hydrogen (*15*) and for the measurement of ethylene production (*16*).

Precautions must be taken to acquire the technique of opening and closing the stopcocks without disturbing the positions of the flasks on the manometers. But, as with other manipulative manometric procedures, only time, patience, and an awareness of the potential defects will permit the successful application of the instrument (*17*).

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26 April 1955

Geologic Application of a Test for Citrate-Soluble Metals in Alluvium

One of us has described a procedure for determining the content of citrate-soluble heavy metals, principally zinc, copper, and lead, in geologic materials (*1*). This is a rapid and simple procedure readily adaptable to use in the field at the collecting site.

Experiments with this test in 1953 in New Brunswick and the Gaspé Penin-

sula of Quebec, Canada, have shown that the citrate-soluble metal content of stream alluvium is a useful method of appraising the possibilities of mineral deposits in the area upstream from the sample site. Samples of alluvium may be taken either from the active bed of the stream or from terrace deposits. Field trials showed the presence of trains of alluvial material containing a higher-than-normal quantity of extractable metal for distances up to 4 mi, and in one case for 50 mi downstream from the deposits. The content of extractable metal depends to a certain degree on the grain size, organic content, and mineral composition of the sample as well as on the distance from the source.

On the basis of these experiments and subsequent experience, regional surveys of the citrate-soluble heavy-metal content of stream alluvium is recommended as a reconnaissance method of mineral exploration.

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21 April 1955

Statistics

As a nonstatistician user of statistical methods, I feel moved to reply to the communication from Frederick Sargent, II, [*Science* **121,** 402 (18 Mar. 1955)]. There is a danger, in my opinion, that the very real case against indiscriminate use of correlation analysis may be stultified by the continued citation of nonsense correlations to support it. That noncausal correlations do exist has already been adequately demonstrated. Offhand I can think of the correlation between birth rate and marriages in the Church of England, and that between stage of the Potomac River and street traffic flow in Washington, D.C., adduced respectively, I believe, by Pearson and Ezekiel.

Actually, the correlations found are but formalizations of common-sense statements: "The summer months, when both precipitation and temperature are high (in Chicago) have shorter names than the others" (the "r" months have provided centuries of oyster eaters with a handy mnemonic); "Both birth rates and church marriages have declined in England"; "Street traffic and river flow have similar diurnal variations."

It would be more useful to students to point out forcibly the need for *a priori* biological hypotheses for correlations to be tested than to produce further examples of noncausal correlations, no matter how amusing.

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11 May 1955

Steroid Anesthetic Agent

Since Selye (*1*) and others reported the central depressant action of certain steroidal hormones, little attention has been given to the quantitative and qualitative comparison of the anesthetic action of steroids and the commonly used anesthetics. The possible use of this type of compound in clinical anesthesia led us to study the relationship of chemical structure to activity in a series of steroids. 21-Hydroxypregnane-3,20-dione sodium succinate (hydroxydione) (*2*) was found to be the most promising of a number of water-soluble steroids. The marked anesthetic action, solubility in water, and lack of endocrine activity and other side effects have justified the evaluation of this compound in man as an intravenous anesthetic (*3, 4*).

On intravenous injection, anesthesia is produced by hydroxydione in mice, rats, cats, dogs, and monkeys (*5*). In the mouse and rat the most striking features of hydroxydione are its remarkably high anesthetic potency, which equals that of Pentothal Sodium (thiopental sodium), and its therapeutic index, which greatly exceeds that of the thiobarbiturate (see Table 1). However, in cats, dogs, and monkeys, hydroxydione is not as active (milligram for milligram) as thiopental sodium is. In dogs, hydroxydione at 100 mg/kg compares in effectiveness with 25 mg of thiopental sodium. However, hydroxydione is much less toxic than thiopental sodium—three of seven dogs died after intravenous injection of 50 mg of thiopental sodium per kilogram of body weight, but only one of eight dogs died after intravenous injection of 300 mg/kg of hydroxydione.

Table 1. Comparative intravenous anesthetic potencies of thiopental sodium and hydroxydione in mice

Compound	AD_{50} (mg/kg)	LD_{50} (mg/kg)	TI (LD_{50}/AD_{50})
21-Hydroxypregnanedione sodium succinate (hydroxydione)	21.5	250	11.5
Thiopental sodium	19.5	80	4

Other advantages of the anesthesia produced are the relatively low degree of respiratory depression and rapid uncomplicated recovery with minimum postanesthetic depression. Little or no endocrine activity can be demonstrated after administration of large doses of hydroxydione to experimental animals (*6*).

In view of the striking pharmacological effects of hydroxydione, it is of interest to reconsider the possible actions of endogenous hormones and their metabolites on the central nervous system, such as in central control of anterior pituitary secretion, where certain analgesics and hypnotics have been shown to exert marked effects. Other central actions of hydroxydione, such as effects on brain metabolism (*7*) and electroshock threshold (*8*), as well as effects in combination with other drugs, are being investigated and will be reported subsequently.

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References and Notes

1. H. Selye, *Endocrinology* 30, 437 (1942).
2. 21-Hydroxypregnanedione acid succinate wat prepared by a synthesis involving the catalytic hydrogenation of desoxycorticosterone followed by succinoylation, mp 195°–197°C; $/a/_D + 95°$ ($CHCl_3$); $\lambda\lambda_{max}^{KBr}$ 5.70, 5.87, 7.01, 7.21, 7.36, 7.68, 8.60, 8.99; M.W. 432; the sodium salt was prepared as a white free-flowing lyophilate, readily soluble in water or mildly alkaline buffer solutions. Viadril[R] brand of hydroxydione is the registered trademark of Chas. Pfizer and Co., Inc., for hydroxydione, 21-hydroxypregnane-3,20-dione sodium succinate.
3. G. S. Gordan *et al.* have studied hydroxydione in a number of patients and have found this agent to be a safe, convenient, and practical basal anesthetic for surgical procedures in man. Report is in preparation.
4. During the course of our clinical studies, a report appeared in which sleep was induced in man by intravenous administration of progesterone [W. Merryman *et al., J. Clin. Endocrinol. and Metabolism* 14, 1567 (1954)].
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23 May 1955