Table 1. Determination of some trace element concentrations in standard granite G-1 and standard diabase W-1 compared with the data of Smales (*5*). The limit of detection for Pb, Sn, Ni, Co is about 2 parts per million.

Element	Concentration (ppm)			
	W-1		G-1	
	This report	Smales	This report	Smales
Cr	105		22	
Pb	n.d.*		48	
Sn	n.d.*		n.d.*	
Zr	125		180	
Ni	110	73	n.d.*	1.0
Co	41	49	n.d.*	2.1

* The element was sought but not detected at the level of sensitivity stated above.

ods of analysis include emission-spectrographic, chemical, x-ray fluorescence, isotope-dilution, and neutron-activation techniques. Of these, the last two generally are considered to be less susceptible to systematic errors caused by matrix effects or contamination.

This paper reports preliminary results obtained for several trace elements by an emission-spectrographic technique which utilizes the buffering action of calcium carbonate. The results on standard granite G-1 and standard diabase W-1 are presented in Table 1 and are meant to add to the accumulating data on these rocks. I make no spectacular claims for accuracy for the elements listed. I feel that the technique employed may be satisfactory in eliminating the effects of varying matrices. This technique has proved to be successful in the analysis of the trace alkaline-earths which are particularly sensitive to matrix composition (*3*). The method has the additional advantage that it can be extended to a wide range of geologic materials ranging in composition from pure silicates through calcareous shales to limestones.

Standards were made by mixing the metal oxides of the elements to be sought in varying amounts in an albite base. The silicate was then mixed with an equal amount of analytical grade calcium carbonate (*4*) which was free of the elements investigated. Correction was made for the high lead content of the albite (90 parts per million). Otherwise, the albite was also free of the elements to be sought.

The procedure of analysis is briefly the following: Five milligrams of each sample are weighed accurately to within 3 percent on a Roller-Smith torsion balance and arced to completion at 16 amp (d-c arc) in deep-cratered electrodes to avoid spattering. Kodak SA No. 1 plates are used. A set of standards is run in triplicate on each plate, and working curves are constructed directly by calculating the intensity of the line of the element sought (with proper background corrections). No internal standard is used. The precision in most cases can be expressed as a coefficient of variation of 10 for a wide range of concentrations. The coefficient of variation increases, however, with decreasing concentration to about 25 for the range around 10 parts per million and lower. This is a common feature of emission-spectrographic analysis.

Comparisons are made in Table 1 for Ni and Co between the values obtained by the technique described above and those of Smales (*5*), who used the method of neutron-activation analysis. It is seen that agreement is not complete for these few samples. It is probably true that neutron activation yields more accurate results, but it is important to have all the variously determined data available. The accuracy of a technique cannot be established by comparisons of one or two samples. Turekian, Gast, and Kulp (*3*) use a method of assessing the accuracy of an emission-spectrographic technique for strontium determination when compared with isotope-dilution analyses. They had seven different rocks to compare.

The reasons for discrepancies in the values reported for standard granite G-1 and standard diabase W-1 among the various investigators and techniques can conveniently be broken down in the following manner: (i) errors in the analyses of G-1 and W-1 due to systematic errors (due to matrix effects) inherent in the scheme of analysis and requiring radical revision of the method to insure accurate results; (ii) errors which are due to ephemeral mistakes such as faulty standard preparation, and so forth, which may give poor results for the one or two samples analyzed. If many samples were compared, then certainly these mistakes would be discovered and rectified; (iii) the possibility of the inhomogeneity of the standard rock powders distributed. Certain trace elements have associations with particular minerals; hence any variation in the relative amounts of the latter will be reflected in the former; (iv) the "accurate" techniques of isotope dilution and neutron activation are also susceptible to systematic errors though of a different kind from those to which the emission-spectrographic or x-ray techniques are susceptible.

In light of the above situation, two suggestions can be made regarding the reporting of comparison analyses. First, the analyst using the emission-spectrographic or x-ray fluorescence technique should exercise caution in his claims for accuracy when he is dealing with complex materials such as rocks. Such claims as those of Hower and Fancher (*6*) for accuracy cannot go unchallenged where there are marked discrepancies between their values and those of other reputable analysts using matrix-sensitive techniques. In addition, there are serious discrepancies with available neutron-activation values that have been reported by Smales (*5*).

Second, it is obvious that comparisons to test accuracy and permit interlaboratory standardization must be made on more than two samples. In the cases of standard granite G-1 and standard diabase W-1, only one figure may be available for comparison, as in the case of Pb, Co, and Ni, because the other rock is below the limit of detection for the emission-spectrograph and x-ray fluorescence methods. Hence no real judgment can be made of the validity of a technique of analysis even with the available comparisons with neutron activation or isotope dilution values.

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Potentiation by Ouabain of Contractile Response of Myocardium to Glucose

Ouabain increases the rate of oxidation of C^{14}-labeled glucose to CO_2 by dog heart slices respiring in Krebs-Ringer phosphate medium, but is without effect on the oxidation of pyruvate (*1*). The significance of this observation with respect to the positive inotropic action of the drug is unknown, for no studies with contracting cardiac preparations have been reported. We have investigated the effect of ouabain on the contractile response of isolated rat ventricle strips to glucose and pyruvate and have obtained results which bear upon the afore-mentioned observations (*2*). Glucose is relatively ineffective by comparison with pyruvate as an energy source for rat myocardium in phosphate medium (*3*), and an increased rate of oxidation of the former might be expected to increase the ability of this substrate to support contractile activity.

Strips prepared from the right ven-

Table 1. Effect of ouabain on contractile response of rat myocardium to glucose and pyruvate.

Substrate	Ouabain concn. (mM)		Percentage of initial amplitude*				
			Minutes after addition of substrate				
			0	5	10	30	60
Glucose	0	(12)†	49 ± 2	36 ± 5	40 ± 5	51 ± 8	59 ± 8
Glucose	0.034	(7)	50 ± 4	57 ± 9‡	60 ± 9‡	74 ± 6‡	81 ± 6‡
Pyruvate	0	(8)	50 ± 3	61 ± 9	76 ± 14	105 ± 10	111 ± 9
Pyruvate	0.034	(6)	51 ± 4	68 ± 8	79 ± 10	97 ± 10	97 ± 14
Substrate-free control	0	(7)	51 ± 1	50 ± 2	48 ± 3	41 ± 3	33 ± 3
Ouabain control	0.034	(6)	50 ± 1	44 ± 4	40 ± 5	30 ± 5	19 ± 4

* Mean values ± standard deviation. † No. of experiments in parentheses. ‡ Significantly different from response to glucose in absence of ouabain [$p < 0.01$ (t test)].

tricle of the rat heart were stimulated in substrate-free phosphate medium at 27°C until the force of contraction had declined to approximately 50 percent of the initial amplitude (*3*). Ouabain was added at this point and, after the positive inotropic action had subsided to the 50-percent level, the substrate under investigation was added. The response was compared with that of strips to which substrate was added at the 50-percent level in the absence of cardiac glycoside. Ouabain increased the force of contraction to approximately 75 percent of the initial amplitude; the force declined again to the 50-percent level in approximately 15 minutes. This transient increase in the force of contraction produced by ouabain appeared to be related to depletion of endogenous substrates and was in marked contrast to the prolonged positive inotropic effect that occurred when the cardiac glycoside was added to slightly hypodynamic preparations (91 percent of the initial amplitude) or to ventricle strips that had become hypodynamic after prolonged contraction in a medium containing glucose. (The positive inotropic response at 37°C has been reported in a previous communication, *4*.)

The responses of the ventricle strip to 5.5 mM glucose in the presence and absence of ouabain are shown in Table 1. A noteworthy difference is seen in the immediate response, with a marked depression of force within 5 minutes in the absence of ouabain in contrast to the steady increase occurring in the presence of the drug. Although the force of contraction subsequently began to increase slowly in the absence of ouabain, the value attained at the end of the experimental period was still considerably less than that attained in the presence of the drug. On the other hand, a steady increase in force which was not influenced significantly by previous exposure to ouabain occurred following the addition of 2 mM pyruvate. Results similar to those with pyruvate were obtained with 2 mM β-hydroxybutyrate (16 experiments).

The fact that glucose was much more effective in sustaining the contractile activity of myocardium in the presence of ouabain than in its absence is in accord with Wollenberger's findings (*1*). It seems clear that the conversion of glucose to pyruvate is affected in view of the failure of the drug to alter the contractile response to pyruvate or β-hydroxybutyrate, or the rate of oxidation of pyruvate (*1*). This finding provides support for the view that the positive inotropic action may be based, at least in part, on an increased energy production by the heart.

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Isolation and Propagation of Rabbit Kidney Epitheliallike Cells

Simplification and refinement of tissue-culture procedures during the last few years have greatly aided viral research studies. Scherer, Syverton, and Gey (*1*) have discussed the need for established cell strains which will support the cultivation of viruses. It is the purpose of this report (*2*) to describe the successful isolation and propagation of a new cell type derived from rabbit kidney tissue (strain RbK). The strain is of potential interest because of its possible susceptibility to poliomyelitis and other viral agents.

On 28 Nov. 1956, both kidneys were removed from an adult, female, New Zealand white rabbit. The capsules were removed and minced kidney cortex (1 to 2 mm fragments) was prepared in Dulbecco's phosphate-buffered saline (*3*). The fragments were washed repeatedly with buffered saline until a clear supernatant was obtained. A cell suspension was prepared from the minced tissue according to the simplified method described by Bodian (*4*). Minced tissue was treated with 0.25 percent trypsin in Hank's balanced salt solution for a total of 24 hours at 5°C and washed twice with balanced salt solution, and the cells were resuspended in 5 ml of growth medium. The latter consisted of Eagle's basal medium prepared in Hank's balanced salt solution, 20 percent rabbit serum and 20,000 units of pencillin, 5 mg of dihydrostreptomycin, and 5000 units of mycostatin per 100 ml. One-tenth-milliliter amounts of undiluted cell suspension and of a 1/10 dilution were added to 0.9 ml of growth medium in Leighton tubes; tube cultures were set up in triplicate. In addition, 1 ml of undiluted cell suspension was added to 4 ml of medium in each of two culture flasks with a surface area of approximately 24 cm^2. All cultures were incubated at 37°C. Seventy-five percent of the culture fluid was renewed every third day.

Microscopic examination of the cultures showed that many cells had adhered to the glass during the first 72 hours of incubation. However, it was observed that growth progressed at a slow rate and was not stimulated by renewal of nutrient fluid. A predominance of spindle-shaped cells was seen in all cultures, and the cells were not in close apposition.

Ten days after the initial planting, the cells in the flask cultures were resuspended at 37°C in 5 ml of Hank's balance salt solution containing 0.25 percent trypsin, centrifuged at 600 rev/min for 10 minutes, and the supernatant was discarded. After resuspension in 5 ml of growth medium, the cells were transferred to clean, sterile flasks and returned to the incubator. No enhancement of growth was observed following this treatment, and the individual cells continued to appear spindle-shaped. On the eighth day the medium was discarded from the culture flasks and replaced with growth medium containing 5 percent bovine embryo extract. The cultures received a 75-percent renewal of nutrient fluid with embryo extract every third day.

Within a week a marked growth response and alteration in cytology were noted. The cells became polyhedral, and populations of contiguous cells were es-