the gas-counting methods and those liquid counting methods which include carbon that does not come from the sample being dated. Furthermore, the benzene method does not require a high vacuum rack or liquid nitrogen, which is not available in most places in the world.

The existing assembly is now capable of providing radiocarbon dates. Work is in progress to set up the operation on a routine basis and to increase the efficiency of the steps in the procedure to increase the yield. It is anticipated that the background can also be reduced considerably (*6*).

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6. Assistance from the Robert A. Welch Foundation of Houston, Tex., is acknowledged.

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Effects of Temperature and Anions on Titration Curves of Frog Muscle

Abstract. Titration curves of frog muscle at 2°C and 25°C were determined in vivo. Lowered temperature decreases respiration without change of colloidal charge or of ion distribution as estimated from charge. Anions which are inhibitors or metabolites combine with muscle colloids, changing the distribution of other anions and cations.

Biological structures are amphoteric colloids which combine with organic and inorganic anions and cations, as well as with hydrogen ions. These ion-binding properties apply both to soluble and insoluble proteins (*1*). They have been studied in titration curves of colloidal surfaces (*2*). Many anions are strongly reactive and have marked effects on the titration curves of proteins and enzymes and on the binding of other anions and cations. The reactive anions include metabolic substrates as well as inhibitors. The effects are especially strong in reactions with insoluble protein aggregates.

Enzymes may occur in solution or as components of insoluble colloidal aggregates (*3*). According to the Michaelis-Menten formulation, combination with ions or molecules conforms to the mass action law, and is a condition for reactions with substrates or inhibitors (*4*). From these considerations, combinations with such substances would produce changes in electrolyte distribution simultaneously with changes in enzyme reactions. Rates of respiration and states of ion distribution both become dependent on the physicochemical state of the colloid and the chemical composition of the environment. It follows that the relation between cellular respiration and ion distribution is not direct, but that it involves colloidal structures of varying physicochemical states.

Experimentally, respiration may be changed by inhibitors or by temperature. In the first case, states of intracellular and extracellular colloids are simultaneously changed. Temperature changes, however, need not change the colloid, especially in *p*H ranges where protein titration curves are nearly independent of temperature. Experiments on the influences of temperature and of 2-4-dinitrophenol on the surface titration curves of frog muscle in vivo are reported here.

Fourteen frogs (*Rana pipiens*) were studied, seven at 25°C and seven in a cold room at 2°C. The frogs were pithed and the anterior thigh muscles were exposed. The overlying perimysium was scraped off to expose the sarcoplasm of the muscle fibres as confirmed by histologic examination. An electrometric method was used to determine the density of charge of the colloidal surface at a series of *p*H's between 7.4 and 2.2 (*5*). This is equivalent to a titration curve of the surface (*2*). The curve was then redetermined with the same series of isotonic saline solutions to which had been added 2-4-dinitrophenol at a concentration of 0.001*M*. The effects of *dl*-lactate, bicarbonate ion, sodium iodoacetate, and sodium cyanide were determined at *p*H 7.4 (Table 1). The compounds were chemically pure or reagent grade preparations.

In the method, described elsewhere in detail (*2*), two potential differences are determined between a reference site and the muscle surface. The reference junction is established by placing one leg in a 0.15*M* NaCl solution, while at the experimental site a solution of buffered 0.15*M* NaCl is applied by means of a moistened cotton pellet. Both reference and experimental junctions are connected through KCl-agar junctions to saturated KCl-calomel half cells, and a potential, E_1, is measured. A 0.015*M* NaCl solution is then applied at the experimental site, and the potential E_2 is measured. The difference $(E_2 - E_1)$ is termed the dilution potential, E_d. The displacement of potential observed with the dilute solution is expressed in millivolts. Depending on the sign of the colloidal charge, it is related to the charge density as follows:

$$E_d = E_d^0 + kx \qquad (1)$$

where x is negative colloidal charge, expressed as equivalents per kilogram of water (above the isoelectric point), or

$$E_d = E_d^0 - ky \qquad (2)$$

where y is the positive charge (below the isoelectric point) expressed in the same way. E_d° is the theoretical dilution potential at zero colloidal charge, and k is a constant which depends on temperature (*6*). Equations 1 and 2 have been derived for amphoteric colloidal surfaces consisting of one or more colloidal components. Potential differences refer to macroscopic sections of the muscle surface of about 1 to 4 mm² (*2*). These contain heterogeneous components of the sarcoplasm.

Results of the potentiometric determinations are presented (Fig. 1, Table 1). Values of x and y at two temperatures are plotted against *p*H to yield titration curves of the surface. The points have been fitted with the same curve, which shows an isoelectric point of about 3.25 (zero x and y). Curves observed with 0.001*M* dinitrophenol have likewise been plotted at two temperatures. These show displacements toward low levels of x; the displacements at 2°C are greater than those at 25°C. They resemble the displacements caused by picric acid at connective tis-

Table 1. Effects of anions on colloidal charge of muscle surface at 25° and 2°C. Change of colloid charge density values are means and change of potential values are means and standard deviations, of four to seven measurements.

Concn.* (*M*)	Temp. (°C)	Change of potential ($E_d - E_d'$)† (mv)	Change of colloid charge density ($x - x'$)‡ (equiv /kg water)
		2-4-Dinitrophenol	
0.001	25	4.4 ± 1.1	0.022
0.001	2	6.2 ± 0.6	.034
		Iodoacetate	
0.001	25	4.0 ± 0.5	0.020
0.01	25	4.6 ± 0.4	.023
0.01	2	6.0 ± 1.3	.030
		dl-*Lactate*	
0.01	25	3.4 ± 0.9	0.017
0.01	2	3.5 ± 0.3	.019
		NaCN	
0.001	25	3.9 ± 0.5	0.020
0.01	25	4.6 ± 0.2	.023
0.01	2	4.7 ± 1.1	.026
		$NaHCO_3$	
0.025	25	3.9 ± 0.3	0.020

* In isotonic NaCl-phosphate buffer at *p*H 7.4. † E_d is control value of dilution potential (−1.2 mv at 25°, and −1.6 mv at 2°). E_d' is the value of dilution potential in presence of given solution. ‡ Value of $(x - x')$, the change of colloid charge density, was calculated from Eq. 1, with the following values of k: 197 at 25°C, and 182 at 2°C.

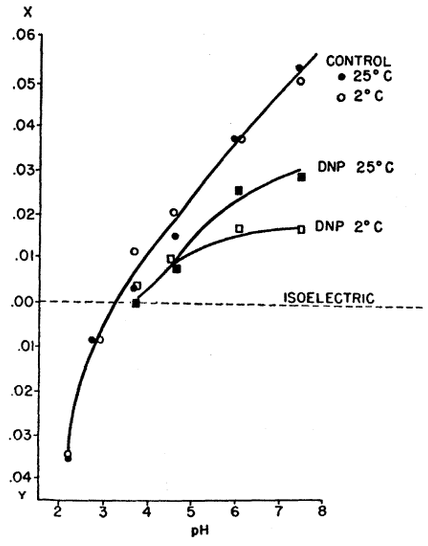

Fig. 1. Titration curves of frog muscle surface. Ordinate, density of colloidal charge in equivalents; abscissa, *p*H. Control curves at 2°C and 25°C are compared with curves observed with 0.001*M* 2-4-dinitrophenol.

sue or wool surfaces (*2*). Similar effects are shown by the other anions (Table 1). In titration curves of wool and connective tissues, many anions combine with protein to displace the curves in the same way.

Certain properties of the muscle surface studied in vivo may be compared with those determined in vitro. The negative charge density at *p*H 7.4 (0.053 equivalents; see Table 1) is lower than that of the isolated muscle proteins (0.100 equivalents) (*7*), but is consonant with that value when bound calcium, magnesium, and amines are taken into account. The fact that anions lower the negative colloidal charge has been discussed elsewhere (*2*). By forming salt linkages with positively charged groups, an anion *A* may increase the negative charge of the colloid *P*. For example,

$$P^{-} + A^{-} = (AP)^{--}$$

If this leads to the simultaneous binding of more than one hydrogen ion, the negative colloidal charge is decreased:

$$(AP)^{--} + 2H^{+} = (APH_2)$$

In the general case *P* would be polyvalent with numerous negative charges. The negative charge would be lowered by the simultaneous binding of anions and hydrogen ions to form the complex (APH_2). In the case of 2-4-dinitrophenol, the anionic O^- group would form a salt linkage with a positively charged amino group of the colloid. The two NO_2 groups would form hydrogen bonds simultaneously. In the process the Donnan ratio is changed, sodium is displaced, and chloride is simultaneously taken up by the surface (*2*). Other anions with strongly electronegative groups (NO_2, I, CN, CHOH) likewise coordinate by means of hydrogen bonds with conjugate electronegative groups (CONH, COO^-, SH, and so forth) of the colloid. In all cases a rearrangement of hydrogen and electrostatic bonds of the colloid would shift the titration curve. Because of the low bond energies which are involved, high labilities would be expected for anion-cation exchange reactions (*2*).

A direct relationship between ion distribution and the energy derived from cellular respiration has frequently been postulated, and appears under various guises as active transport of ions. The above experiments show that redistribution of anions and cations is produced by combination of respiratory inhibitors with extra- and intracellular charged colloidal aggregates. Anionic metabolites and inhibitors similarly alter the titration curves (and ion distributions) of connective tissues, as well as those of metabolically inert wool fibers and other colloids including ion-exchange resins. Lowering of respiration in poikilotherms by temperature reduction leads to no significant effect on colloidal charge or electrolyte distribution, although energy production is greatly diminished.

From this point of view, respiratory changes without simultaneous changes in colloidal state need not affect ion distribution. Inhibitors, which by definition decrease respiration, are bound to the colloidal matrix, and thereby produce redistributions of all other anions and cations (*8*).

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5. Buffer solutions were prepared at six different *p*H levels: 7.4, 6.0, 4.6, 3.7, 2.9, and 2.2. The ionic strength in each case was approximately 0.15. At *p*H 6 and 7.4, phosphate buffers were used, while acetate–acetic acid mixtures were used at *p*H 3.7 and 4.6. Sodium was the only cation present, while chloride accounted for 90 percent or more of the total anion concentration. NaCl-HCl mixtures were used at *p*H 2.9 and 2.2.
6. Below *p*H 3 the values of $Ea°$ depend on *p*H as well as on temperature. At 25°C the values of $Ea°$ in millivolts are −11.8 (*p*H 3.7 to 7.4), −10.1 (*p*H 2.9), and −4.2 (*p*H 2.2). At 2°C the corresponding values are −10.9, −9.3 and −3.9 mv. The values of *k* are 197 (25°C) and 182 (2°C). Methods of calculating these constants have been given (*2*).
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Cranial Capacity of Oreopithecus bambolii

Abstract. From a plaster reconstruction of the skull of the August 1958 skeleton, the cranial capacity of *Oreopithecus bambolii* has been estimated as falling between 276 and 529 cubic centimeters, thus within the ranges of variation of both orangutan and chimpanzee. In cranial capacity, therefore, and probably in body-brain ratio as well, *Oreopithecus* is a hominoid.

The taxonomic status of *Oreopithecus bambolii,* a fossil catarrhine primate from the Lower Pliocene of Italy, hence some 12 million years old, has been a matter of considerable controversy ever since the type specimen, a mandible with teeth, was described by Gervais in 1872 (*1*). In recent years, however, particularly following Hürzeler's discovery of a large number of additional specimens at Baccinello, Italy, it has become increasingly evident that *Oreopithecus* is a member of the superfamily Hominoidea, which comprises the families Pongidae (anthropoid apes) and Hominidae (man and his immediate forerunners) (*2*). This interpretation has been strengthened by studies of an adult skeleton discovered on 2 August 1958 (*3*). The precise allocation of *Oreopithecus* within the Hominoidea remains uncertain, however. The present communication deals only indirectly with this problem. Rather, it deals specifically with an attempt to estimate the cranial capacity of the 1958 skull, which is the only one sufficiently complete to justify such a procedure.

Although this skull is considerably crushed, its fragments fortunately remained closely associated within its lignite matrix. Its condition, however, precludes direct measurement of the cranial capacity. Some indirect method therefore must be employed. While we were in Basel in August, 1959, Dr. Hürzeler generously permitted us to study his plaster reconstruction of the skull and to take the basic measurement necessary for an estimation of its cranial capacity (*4*). This consists of an esti-