South America and Australia are very impressive. They leave no doubt in the reader's mind of the magnitude and certainty of the refrigeration. Continental ice sheets of Silurian age are known in Alaska. The Varanger Fiord tillites of northern Norway have been recently correlated with the Ordovician. Widespread Ordovician glacial conglomerate is also recognized in Gaspé. Early Cambrian or late pre-Cambrian tillites are described from the Yangtzi Canyon in China, from southern Norway, from several places in North America, from Africa and from Australia, placing this ice age as equal in magnitude to that of the Pleistocene.

The widespread Gowganda tillite of Huronian age in Canada is convincingly described as of glacial origin. And beds of similar age, probably of glacial origin, are cited from several other parts of the world. Conglomerates strikingly like tillites are discussed from the Timiskamian and Keewatin rocks of Canada, though metamorphism has rendered their sure reference to glacial origin uncertain.

The effects of glacial periods on both plants and animals, treated rather fully, makes a very suggestive contribution to the study of organic evolution.

Defects in all existing theories of the cause of ice ages are pointed out. But the author confesses he is unable to propose something better. In his opinion the solution must come from general and local causes in a combination of astronomic, geologic and atmospheric conditions.

Photographs are numerous and in general very good, though many of them lack a scale. The book is of great importance not only to the glacial geologist, but to the historical geologist, the paleontologist and the paleobotanist as well.

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SPECIAL ARTICLES

THE NATURE OF THE "INORGANIC PHOSPHATE" IN VOLUNTARY MUSCLE

SOME months ago we[1] described a colorimetric phosphate method, the special feature of which is the use of a very active agent (aminonaphtholsulfonic acid) for converting the phosphomolybdic acid to its blue reduction product. When we first made use of this method for the determination of inorganic phosphate in protein-free muscle filtrates, shortly after the details had been worked out, we found a marked delay in color production. The time required to reach a constant reading was about thirty minutes, whereas ordinarily the full color (relative to the standard) has developed within four minutes or less. This peculiar behavior appeared to indicate that muscle contains either some substance capable of retarding the color reaction or else a very unstable (presumably organic) compound which liberates o-phosphoric acid while the color is developing. While the course of the color development in muscle filtrates turned out to be quite different from anything which we had seen in testing out the method in the presence of known interfering substances, it is impossible to rely on this point as a means of distinguishing between the two alternatives. Ferric salts, for example, also behave in a way that is unique. A mixture of inorganic phosphate and ferric chloride certainly does not contain a highly unstable organic phosphorus compound, and the delayed color reaction found with muscle filtrates therefore does not constitute conclusive proof of the existence of such a substance in the muscles.

Further study of the course of color development nevertheless did bring out some interesting and suggestive points, notably the fact that the delay is hardly any more pronounced with 10 cc of muscle filtrate, for example, than with 5 cc or less. Every interfering substance which we investigated in the course of our work on the phosphate method, on the other hand, shows a much more marked effect when the phosphorus content of the sample is increased. Although these facts have been in our possession now for more than a year, we have until this time refrained from placing them on record, inasmuch as the phenomena observed could not with any certainty be ascribed to the presence of an organic compound of phosphoric acid until the compound had been isolated, or at least until the organic radicle had been identified. Both these things have now been done, although the isolated substance has not yet been obtained in the pure state, and the outcome appears likely to throw light on a field of biochemistry never before suspected of being in any way related to phosphoric acid.

Muscle filtrates from which all the inorganic phosphate has been removed (by precipitation with barium, silver, etc.), as well as material which has been still further purified, show the same delay in the production of the color. These facts, together with the knowledge that the delayed reaction really is associated with the hydrolysis of an organic compound of phosphoric acid, give real significance to the quantitative data which we have meanwhile been accumulating. Some of these data will now be presented before we proceed to a discussion of the nature of the substance.

The method which we have used for the determination of this unstable form of phosphorus (which we shall for the present designate as "labile phosphorus")

[1] C. H. Fiske and Y. Subbarow, *J. Biol. Chem.*, Vol. 66 (375)—1925.

differs in no respect from our regular phosphate method, except that readings are taken at brief intervals (every minute, beginning with the third, until the unstable substance is about half hydrolyzed, and thereafter every few minutes until no further change occurs). The growth in color intensity for the first few minutes is practically linear, and the concentration of inorganic phosphate is found by extrapolating back to zero time. The sum of the inorganic and the "labile" phosphorus is calculated from the final reading, and the amount of "labile phosphorus" found by difference. From control analyses of solutions of the purified organic compound, with and without the addition of known amounts of inorganic phosphate, we believe that the results are accurate within 1 or 2 mg of phosphorus per 100 gm of muscle.

The principal results which we have obtained by this method of analysis are as follows: (1) The normal resting voluntary muscle of the cat shows generally about 60 to 75 mg of "labile phosphorus" per 100 gm if removed with the greatest possible care and at once cooled to 0° C. or below. (The further preparation of the material for analysis consists simply in precipitating the protein with ice-cold trichloroacetic acid, filtering and immediately neutralizing the filtrate with sodium hydroxide.) The true inorganic phosphorus under these conditions is only 20 to 25 mg, instead of the 80 to 100 mg, shown by other methods of analysis. (2) After prolonged electrical stimulation, the "labile phosphorus" usually falls to about 20 mg (in one instance to as little as 9 mg), while the inorganic phosphorus is correspondingly increased. (3) Stimulation with the blood supply shut off, in which case complete fatigue ensues, causes complete disappearance of the unstable compound, within the error of analysis. Merely shutting off the circulation for an equal length of time has little or no effect. (4) A period of rest after the muscle has been stimulated is accompanied by resynthesis of the organic compound. The maximum yield of "labile phosphorus" so far observed under these conditions is 46 mg per 100 gm of muscle. The inorganic phosphate, however, falls to the normal level, so in all probability either some of the phosphate has been discharged into the circulation, or else the water content of the muscle has increased.

The compound under consideration is a derivative of creatine. On a small scale we have succeeded in separating it from all other phosphoric acid compounds. On a sufficiently large scale to yield material enough for a complete analysis, the separation is not so readily accomplished by our present methods, and the best products that we have so far procured contain several per cent. of one or more other phosphorus compounds, since the full blue color can not be obtained unless the material is ashed. In spite of this contamination, the phosphate, creatine and base in two salts which have been prepared add up to about 85 per cent. of the weight of the entire substance, indicating the probable absence of a third component. The elementary analysis of amorphous products, unless they are of definitely established purity, proves very little. Our main evidence for the existence of "phosphocreatine" in muscle is of a quite different nature. In the first place, we have found that the "labile phosphorus" can be more or less completely separated by precipitation with a number of different reagents (six in all have been used to date), and that in each case there is precipitated with it one equivalent of creatine. One of these reagents (copper in slightly alkaline solution) has been applied to muscle filtrates prepared under various conditions, and the proportionality between creatine and "labile phosphorus" has never failed. A few typical illustrations of our experience with copper precipitation are given in the accompanying table. They serve to show that creatine and the labile form of phosphate are precipitated together, in equivalent proportions, whether the muscle was fresh and in the resting state or whether it had been subjected to manipulations which alter the concentration of the "labile phos-

	Mg per 100 gm muscle		
	"Labile phosphorus"	Creatine in washed copper precipitate	Molecular ratio of creatine to phosphoric acid
Normal resting muscle	70	280	0.95
do	77	323	0.99
Normal resting muscle:			
(1) Fresh	65	269	0.98
(2) Stood few minutes after removal from body	55	222	0.96
(3) Stood for longer time	32	128	0.95
(4) Analysis of trichloroacetic acid filtrate (from fresh muscle) which had stood for several hours	0	2	
Muscle stimulated for 10 minutes with circulation intact	20	82	0.97
Muscle stimulated to fatigue with blood supply shut off, followed by 1 hour rest period	44	192	1.03

phorus." Thus, when the muscle is allowed to stand outside the body, the "labile phosphorus" content progressively diminishes at a fairly rapid rate (it is entirely gone in less than twenty minutes), and at the same rate creatine is set free, as indicated by its failure to be thrown down by copper. If the trichloroacetic acid filtrate prepared from a sample of fresh muscle is kept (unneutralized) for several hours, the "labile phosphorus" has completely disappeared, and the copper precipitate is then virtually free from creatine. The same parallelism holds for muscle which has been stimulated, showing that the liberation of inorganic phosphate during stimulation is associated with the conversion of the creatine to a form in which copper will not precipitate it. Finally, in case the muscle has been stimulated with the blood supply cut off—a procedure which, as stated, leads to the loss of all the "labile phosphorus"—and then permitted to recover, the reappearance of "labile phosphorus" is accompanied by the return of an equivalent quantity of creatine to the condition in which precipitation by copper does take place. Direct evidence for the synthesis of a creatine-phosphoric acid compound during recovery is thereby attained.

Quite aside from its obvious bearing on the mechanism of muscular contraction, the demonstration of "phosphocreatine" in muscle should go far towards providing an explanation for a number of matters which in the past have been obscure. Among these may be mentioned the passage of administered creatine into muscle in spite of the large quantity already there, and the striking difference between resting (living) muscle on one hand and fatigued or dead muscle on the other in their capacity for retaining both creatine and phosphate, as shown by perfusion and dialysis experiments.

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ON THE UPPER LIMIT OF VIBRATIONAL FREQUENCY THAT CAN BE RECOGNIZED BY TOUCH

IN relation to experiments on the transmission of speech instrumentally to organs of touch in the skin and to end organs of the sense of vibration it is of more than ordinary interest to discover what is the upper limit of vibrational frequencies that can be felt through these senses.

Landlois (*Lehrbuch d. Physiol. d. Menschen,* 1880) states that vibrations of strings are recognizable at a frequency of 1552 a second. This is the highest figure that has been published hitherto.

Dr. V. O. Knudsen, of the Department of Physics of the University of California, reports in a paper that is about to be published that his subjects reached no higher than 1600 d.v. a second. The stimulus was furnished by an oscillator and was applied through a reed slapping near the tip of the subject's middle finger.

In my experiments the stimuli are the vowel qualities *e, er, oo, o, ah* and *aw,* spoken into a high-grade microphone. A modified 25 B (Western Electric) two tube amplifier is in circuit. An electrical filter has been introduced also, and a five unit receiver or teletactor. Each of these instruments has been made for my use by the Bell Telephone Laboratories. The filter analyzes the speech frequencies into five bands: 0–250; 250–500; 500–1000; 1000–2000 and 2000 plus vibrations a second. Each band is led into one unit in the receiver and into no other. In the course of the experiments that are reported here connections have been broken so as to eliminate all but the highest band of frequencies—2000 d.v. and above.

When the vowel qualities already mentioned are pronounced into the microphone the subject feels the fifth reed that remains connected through the filter when it is in vibration. The figures below indicate the degree of the subject's accuracy of detection. He reported "yes" when he felt a vibration; otherwise he was silent. The stimulus is received through the finger nail or through the skin—preferably the former.

Total impressions—75
C. Subj.; G. Exp.

e	total	19	Vibrations.	Felt	15
er	"	13	"	"	0
oo	"	12	"	"	1
o	"	8	"	"	0
ah	"	10	"	"	3
aw	"	13	"	"	6
		75			25

O. Subj.; G. Exp.
Total impressions—74

e	total	18[1]	Vibrations.	Felt	18
er	"	13	"	"	7
oo	"	12	"	"	12
o	"	8	"	"	2
ah	"	10	"	"	3
aw	"	13	"	"	11
		74			53

The subjects in this test are both deaf. They can not hear speech. The experimenter, while the test

[1] Missed one *e*; finger off receiver.