It was found that neither of the antisera against types I and II and strains No. 9731 and No. 2-193 agglutinated these organisms to more than a very small fraction (1% or less) of the titers. Likewise, the suspensions of the *S. alkalescens* strains were not agglutinated or agglutinated only to a fraction of the titers of antisera against the typhoid-paratyphoid organisms. It is evident, therefore, that the 4 types of *S. alkalescens* do not share any major antigens with the typhoid-paratyphoid organisms studied.

Summary and Conclusions. Two hitherto undescribed antigenic types of *S. alkalescens* have been reported. The representative strains have the morphological, cultural, and biochemical characteristics of *S. alkalescens* type I and do not share major antigenic components either with each other or with types I and II. One of the strains was isolated by Captain Ewing, the other at this laboratory. At the present time, therefore, *S. alkalescens* comprises 3 antigenic types, namely, type I composed of 4 sub-types, and the 2 new types described here. It is doubtful whether type II of Assis will ultimately be classified as *S. alkalescens.*

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Effect of Lowered Blood Supply and of Glucose-1-Phosphate on Healing of Bone Fractures.*

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Promotion of bone fracture healing is a serious problem at all times in the advanced age group of the population, because, with advancing age, there is an increased susceptibility to fractures, and difficulty and delay in their healing is a fairly common occurrence. The study of therapeutic measures to promote fracture healing in older individuals has been retarded up to now by lack of a method of producing a delay in the union of fractures comparable to that found in old age.

In this communication there is described a simple experimental procedure which yields a long delay in the healing of bone fractures. The technic consists simply of ligaturing the femoral artery of a limb and thereby reducing the blood supply to the affected bone. By this technic it is hoped that therapeutic measures can be tested which eventually will prove to be of benefit in the treatment of delayed union in the aged. The results obtained on rats by this technic will be presented below.

The high alkaline phosphatase activity of calcifying bone has attracted the attention of investigators since the pioneer work of Robison. Robison's theory[1] that the phosphatase acted to raise the product of calcium and phosphate ions by liberating inorganic phosphate from organo-phosphates in the blood faced the difficulty that the acid-soluble organic ester content of the blood plasma is small. It has been observed that glycogen increases in hypertrophic cartilage prior to ossification and disappears during ossification (Harris,[2] Glock[3]). The presence of the en-

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1 Robison, R., *The Significance of Phosphate Esters in Metabolism,* 1932, New York Univ. Press, N.Y.

2 Harris, H. A., *Nature,* 1932, **130**, 996.

3 Glock, G. E., *J. Physiol.,* 1940, **98**, 1.

zyme phosphorylase in hypertrophic cartilage has been demonstrated by Gutman and Gutman.[4] These observations lend themselves to a theory of bone calcification which is represented by the equations given below.

$$\text{Glycogen} + \text{phosphate} \xrightarrow{\text{phosphorylase}} \text{Glucose-1-phosphate}$$

$$\text{Glucose-1-phosphate} \xrightarrow{\text{phospho-glucomutase}} \text{Glucose-6-phosphate}$$

$$\left.\begin{matrix}\text{Glucose-1-phosphate}\\ \text{Glucose-6-phosphate}\end{matrix}\right\} \xrightarrow{\text{bone phosphatase}} \text{Glucose} + \text{phosphate}$$

The above reactions show that bone phosphatase would, as suggested by the theory of Robison, liberate inorganic phosphate and tend to cause a supersaturation of the bone salts. However, according to the above equations, the substrate for the action of the phosphatase is formed in the ossifying cartilage directly and is not brought to the bone by the circulation.

Although bone phosphatase can hydrolyze either of the glucose phosphate esters shown above, not only they, but some further product in the phosphate cycle of carbohydrate breakdown could equally well serve as the substrate for bone phosphatase activity. In the mechanism of the regulation of the blood glucose level by the liver it is postulated that glucose-6-phosphate is the glycogen product which is hydrolyzed by liver phosphatase to glucose and inorganic phosphate (Ostern and Holmes,[5] Cori and Cori[6]).

A corollary which follows from the theory advanced above, is that administration of the proper organo-phosphate should promote bone fracture healing. The effect of β-glycerol phosphate on fracture healing has been tested by Armstrong, Sperling, and Letow,[7] who believe they observed some favorable effect. However, unfortunately, the experiments of the above authors were not controlled by tests of the effect of the administration of an equivalent dose of inorganic phosphate.

In the present investigation preliminary tests of the effect of glucose-1-phosphate (Cori ester) on bone fracture healing have been carried out. The ester was synthesized with phosphate labeled with P^{32} so that the fate of its contained phosphate could be followed by the tracer technic. Negative results were obtained when the ester was injected subcutaneously. It is planned to continue the tests on the therapeutic effects of this and of other organo-phosphates.

Experimental Methods and Results. 1. *Lowered Blood Supply and Fracture Healing.* The left femoral artery was exposed and ligatured lightly so as to diminish but not completely stop the blood flow. While under the anesthetic, both the right and left fibula were fractured with the special forceps designed by Hertz.[8] The rats were sacrificed at intervals of 6, 12, 13, 18, 24, and 30 days after the operation. Each animal was given an intraperitoneal injection of 0.2 mg Sr^{89} in physiological saline solution 48 hours before being sacrificed. The fibulæ were then dissected out and tested for Sr^{89} activity and breaking strength as described elsewhere (Copp and Greenberg[9]). The results are summarized in Table I. It will be observed from the table that the reduction in the rate of bone healing caused by the lowered blood supply is indeed striking.

2. *Effect of Glucose-1-Phosphate on Fracture Healing.* The glucose-1-phosphate was synthesized by the action of the enzyme phosphorylase on glycogen and a solution of inorganic phosphate containing P^{32} (McCready and Hassid[10]). The tests were carried out on rats with the left femoral artery ligatured as described above. From the fifth day after fracture of the fibulæ, the animals were in-

[4] Gutman, A. B., and Gutman, E. B., PROC. SOC. EXP. BIOL. AND MED., 1941, **48**, 687.

[5] Ostern P., and Holmes, E., *Nature*, 1939, **144**, 34.

[6] Cori, G. T., and Cori, C. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 337.

[7] Armstrong, W. D., Sperling, L., and Litow, S., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 169.

[8] Hertz, J., *Acta pathol. microbiol.* Scand., 1936, Vol. 26-30, Supplement.

[9] Copp, D. H., and Greenberg, D. M., *J. Nutrition*, in press.

[10] McCready, R. M., and Hassid, W. Z., *J. Am. Chem. Soc.*, 1944, **66**, 560.

TABLE I.
Effect of Lowered Blood Flow on the Rate of Healing of Bone Fractures.

	Ligatured			Control		
Time days	No. rats	Sr^{89} uptake % of dose $\times 10^4$	Breaking strength, g	No. rats	Sr^{89} uptake % of dose $\times 10^4$	Breaking strength, g
6	7	3.73 ± 0.50	0	7	5.36 ± 0.66	0
12	7	6.85 ± 1.00	56 ± 40	7	12.78 ± 0.70	420 ± 160
13	4	7.80 ± 0.80	70 ± 35	4	16.00 ± 1.80	615 ± 95
18	7	9.30 ± 1.54	81 ± 36	7	25.35 ± 3.22	686 ± 200
24	7	8.86 ± 2.64	93 ± 55	7	14.80 ± 2.64	593 ± 150
30	4	7.26 ± 0.40	270 ± 100	4	9.27 ± 0.40	890 ± 20

Measure of variability is mean deviation.

TABLE II.
Effect of Subcutaneous Administration of Glucose-1-Phosphate on Bone Fracture Healing.

	Ligatured		Not ligatured	
No. rats	P^{32} uptake, % of dose	Breaking strength, g	P^{32} uptake, % of dose	Breaking strength, g
		Glucose-1-Phosphate.		
5	1.13 ± 0.17	50 ± 40	1.69 ± 0.42	265 ± 40
		Inorganic Phosphate.		
4	1.32 ± 0.19	30 ± 40	2.40 ± 0.56	270 ± 60

Measure of variability is mean deviation.

jected twice daily subcutaneously with 1 ml of solution containing 0.1 g of the potassium salt of the Cori ester for a period of 5 days. Control animals were similarly injected with 0.5 ml solutions containing an equivalent amount of inorganic phosphate. On the eleventh day the animals were sacrificed and the fibulas tested. The results are given in Table II.

In these experiments there was no observable difference in effect between the glucose-1-phosphate and the inorganic phosphate. Neither reagent noticeably accelerated the healing of the bone with the reduced blood supply. However, there is no assurance that the intact ester reached the fractured bone. Prior hydrolysis by phosphatase may have occurred. More direct means of perfusing the bone with the ester will have to be carried out to make the results conclusive.

Summary. 1. A long delay in bone fracture healing can be produced by reducing the blood supply to the affected bone. This technic is suggested for the study of therapeutic measures to promote the healing of delayed union of bone in the aged. 2. Glucose-1-phosphate, when administered by subcutaneous injection, had no favorable effect on the healing of bone fractures.