TABLE I.
Production of Gas in Salicin Medium of Variants of *E. anindolica* G.

No. of transplant	Daughter colonies									
	1	2	3	4	5	6	7	8	9	10
1	0*	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0
6	5	10	0	20	10	0	0	0	0	5
7	10	10	5	25	15	0	0	0	0	15
8	10	10	5	25	30	0	0	0	20	10
9	20	20	15	25	25	0	20	0	25	20
10	20	25	25	20	15	5	15	0	20	15
11	20	25	35	20	15	20	25	0	20	30
12	30	20	30	20	15	20	20	20	20	35
13	35	35	30	25	15	20	20	30	20	40
14	35	35	20	25	15	30	25	35	20	40
15	35	35	30	30	25	30	30	35	35	40

* Figures are in percentages of gas produced in 7 days.

positive variants.

It was discovered that the salicin-positive variants developed a greater capacity to form acid from salicin. The original cultures (salicin-negative) showed an acid production for about 10 hours to a minimum pH of about 6.7, after which there was an abrupt rise in pH to over 8.5 during 180 hours. The salicin-positive variants from these cultures reduced the pH value of the medium to a value around 5 in 10 to 30 hours. The acid production was so great that the organisms were killed in most instances.

The results of this investigation indicate that, the fermentation of a given substance, such as salicin, is not necessarily a constant characteristic. Because of the acquired ability to ferment this glucoside, a number of organisms changed from one species to another according to the Bergey classification. Five cultures change their classification from *E. anindolica* to *E. communior*, 3 from *E. formica* to *E. coli*, and 3 from *E. gruenthali* to *E. paragruenthali*.

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High Molecular Weight Polyglycols as Reagents in the Gravimetric Determination of Inorganic Phosphate.

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Despite the availability of the simpler, direct colorimetric methods for the determination of small amounts of inorganic phosphate in biological materials, gravimetric methods, by reason of their greater precision and accuracy, have continued to find some application. Two precipitates that have been employed for quantities of phosphate too small for the magnesium ammonium phosphate method are the ammonium and strychnine salts of phosphomolybdic acid. The strychnine phosphomolybdate appears to have gained wider preference; its advantages over the ammonium salts have been summarized by Peters and Van Slyke[1] as follows: (1) the weight of the precipitate is greater, for per 1 mg of P the

[1] Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry*, Vol. II, Methods, pp. 849, 873, Baltimore, The Williams and Wilkins Co., 1932.

ammonium phosphomolybdate weighs 69 mg and the strychnine phosphomolybdate 89.3 mg; (2) the strychnine precipitate is more insoluble and forms much more rapidly; (3) the strychnine precipitate readily coagulates into large particles which can be more easily handled than the ammonium precipitate and have less tendency to adhere to the walls of the precipitating vessel; and (4) the strychnine precipitate can be dried to constant weight in an ordinary drying oven at 100° to 110°.

With the discovery that the solid polyethylene glycols form highly insoluble complexes with phosphomolybdic acid in the presence of barium,[2] the possibility suggested itself that these compounds might be adapted to a gravimetric determination of phosphate even more advantageously than strychnine. Subsequent experimentation has justified this expectation, and the present report describes the results obtained with the substitution of a high molecular weight polyglycol for strychnine in the Embden-Fetter method for the determination of inorganic phosphate in blood and urine. The barium-polyglycol-phosphomolybdate precipitate is the equal of strychnine phosphomolybdate on each of the last 3 counts mentioned above, and is superior in that it weighs 97.1 mg per mg of P. Its sole disadvantage is that its formation requires the presence of barium and, conversely, the absence of sulfate. This interference, however, may readily be eliminated by a precipitation preceding the filtration that accomplishes deproteinization of the sample. Minor considerations in favor of the use of the polyglycols are their low cost and availability since they are synthetic chemicals produced in tonnage capacity.

Reagents. All reagents employed, with the exception noted, are those specified in the Embden-Fetter method.[1] The polyglycol used is a commercial product sold under the trademark* name of "Carbowax" compound 6000, the numeral indicating a mixture of polymers with a mean molecular weight of 6000. As purchased, this material contains a small quantity of phosphate as an impurity, which may be removed by adsorption on basic ferric acetate in the following manner: Twenty g of the polyglycol are dissolved in *ca.* 160 ml of water and the solution is made faintly acid to methyl red by the addition of a few drops of dilute acetic acid. Then there are added in succession 4 ml of a 10% solution of ferric chloride in 0.2 N hydrochloric acid and 8 ml of a 5% solution of ammonium acetate. The mixture is heated with continual agitation until boiling just begins, at which point it is cooled momentarily and a few drops of dilute ammonium hydroxide are added. The voluminous precipitate which forms is immediately filtered. The filtrate, which is the polyglycol solution to be employed in subsequent work, should be water-white or of only a very faint greenish color, and should give no turbidity upon mixing with the molybdate solution. The precipitating reagent is prepared within an hour of use by mixing one volume of the polyglycol solution with three volumes of the molybdate reagent, in the same manner as the strychnine molybdate is prepared.

No sulfate was encountered in either of the two samples of "Carbowax" compound 6000 used in this work; however, had this anion been present, it would obviously have been necessary to have removed it prior to employment of the reagent. This removal could probably be best effected by acidifying the original polyglycol solution, adding barium chloride, and filtering with suction through fine asbestos. The reaction of the filtrate would then be adjusted with dilute ammonium hydroxide, and the removal of phosphate carried out from that point.

Procedures. (A) For Blood. Five ml of blood, plasma, or serum are measured into a 50 ml flask containing 25 ml of 10% trichloracetic acid. Following a thorough shaking of the coagulum, 5 ml of a 10% barium chloride solution are added, and the flask is filled to the mark with water. After about 15 minutes of standing, the contents are filtered through Whatman No. 42 or comparable paper. Twenty-five ml of the filtrate are diluted to 60 ml, and 20 ml of the polyglycol-molybdate solution are added. The mixture is allowed to stand at room temperature for one hour

[2] Shaffer, C. B., and Critchfield, F. H., *Ind. Eng. Chem., Anal. Ed.*, 1947, in press.

* Carbide and Carbon Chemicals Corporation.

TABLE I.
Comparison of Phosphate Determinations on Random Samples of Plasma and Urine Performed by the Reference Method and Duplicated by the Proposed Method.

	Phosphate P per 100 ml			
	Plasma		Urine	
Sample No.	Strychnine mg	Polyglycol mg	Strychnine mg	Polyglycol mg
1	5.15	5.36	79.14	80.55
2	3.72	3.72	115.12	117.52
3	4.37	4.15	4.07	4.02
4	3.52	3.44	69.41	68.91
5	4.44	4.64	27.42	28.02
6	3.52	3.52	56.49	57.06
7	3.56	3.52	169.83	172.21
8	2.82	3.08	32.08	32.14
9	3.78	3.60	70.71	71.07
10	2.90	2.84	109.67	109.18
11	3.38	3.52	29.85	30.38
12	2.90	3.04	77.26	79.00
13	4.68	4.92	86.02	85.49
Mean	3.75	3.80	71.31	71.96

with occasional shaking to complete the precipitation. The precipitate is then filtered in a small Gooch crucible previously dried at 110°, cooled in a desiccator, and tared. It is important to use only gentle suction during filtration. Particles of the precipitate which adhere to the container are rinsed into the crucible with 25 ml of ice-cold, diluted (1:5) molybdate solution. The precipitate is then washed with ice-cold water until the washings are no longer acid. During the washing, as soon as one portion of fluid has been drawn through the crucible another portion is added or else the suction is stopped, to prevent the precipitate from becoming divided by cracks through which subsequent portions of wash water can run with little contact with the precipitate. The crucible is dried for an hour at 100°-110°, cooled in a desiccator, and weighed.

(B) For Urine. Five ml of urine, well mixed to suspend any insoluble phosphates, are measured into a 50 ml volumetric flask together with 10 ml of water and 25 ml of 10% trichloracetic acid. Five ml of a 10% solution of barium chloride are then added dropwise, and the flask is allowed to stand undisturbed for one-half hour. At the end of this time the contents are shaken and made up to the mark with water. The barium sulfate precipitate, together with any coagulum in the flask, is separated by filtration, or by centrifugation if necessary, and an aliquot of the clear filtrate (usually 10 to 20 ml depending upon phosphate content) is diluted to 60 ml with water, 5 ml more of the barium chloride solution are added, and the rest of the determination is carried out as described above for blood.

(C) Determination of gravimetric factor. Just as in the case of the strychnine precipitate, each analyst should determine for himself the factor indicating the mg of P per mg of barium-polyglycol-phosphomolybdate precipitate. It has been found that different preparations of strychnine may give different weights of precipitate per mg of P, and it may be assumed that less homogeneous polyglycols will show some limited variation in this respect, although samples from 2 different production batches tested in this work gave identical results. For determination of this factor, a standard phosphate solution containing 0.4395 g/l of thoroughly dried reagent grade KH_2PO_4 was prepared. Aliquots containing 0.5 to 1.5 mg of P (5 ml to 15 ml of solution) were measured from a certified buret, diluted to 60 ml with water, 5 ml of barium chloride solution were added, and the phosphate was precipitated and weighed in the manner described. From a series of 15 trials the gravimetric factor was calculated

as 0.0103.

Results. Table I lists the results of inorganic phosphate determinations of 13 different samples of dog plasma and 13 different samples of human urine carried out by the proposed method, as compared with duplicate determinations performed simultaneously on the same specimens by the Embden-Fetter strychnine-phosphomolybdate method. Statistical treatment of these values by the application of the t distribution indicated no significant difference between the results obtained.

In the analysis of whole blood, correlation of the results obtained by the two methods on a given sample is good provided that filtration of the precipitate is performed promptly at the conclusion of the one-hour period of standing recommended. However, when this time of standing is prolonged, hydrolysis of organic phosphate appears to take place more extensively in the strychnine-precipitated sample and to make the results from this procedure high as compared with those from the other.

Summary. A method is described for the precipitation of phosphate phosphorus as a barium-polyglycol-phosphomolybdate complex which may be filtered and dried to constant weight at 100°-110°. Evidence is presented to show the applicability of this reaction to phosphate determinations on biological materials, and a comparison is made of this procedure with the strychnine phosphomolybdate (Embden-Fetter) method.

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Effects of Vital Dyes on Early Development of the Amphibian Embryo.

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A vital dye may be used to stain different cells and regions of eggs and embryos. To be successfully employed in this fashion, the dye should be "non-toxic and harmless so as not to destroy or even impair the vital activities and embryonic performance of its carrier" (Weiss[1]). However, it has been shown by Child and his co-workers,[2] in the work on the occurrence of physiological gradients, that so-called "vital" dyes, especially the basic ones, can be quite toxic. Determination of the conditions under which vital staining of eggs and embryos can be successfully accomplished is of interest. The general effects of two basic dyes, Nile Blue Sulfate and Neutral Red, were studied by Detwiler[3] in Amblystoma embryos. Gersch[4] and Gersh and Ries[5] in their work on sea-urchin eggs, found that different concentrations of the acid dye, Trypan Blue, caused severe developmental anomalies. Inhibition of mitosis was shown to occur in the larvæ of *Triton taeniatus* after a one- to two-hour exposure to certain concentrations of Neutral Red (Luther[6]). However, Uschin[7] who studied the same problem in *R. temporaria* tadpoles found no dye effects on mitosis.

In view of the frequency with which the

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1 Weiss, P., *Principles of Development*, 1939, Henry Holt and Co., New York.

2 Child, C. M., *Patterns and Problems of Development*, 1941, The University of Chicago Press.

3 Detwiler, S. R., *Anat. Rec.*, 1917, **13**, 493.

4 Gersch, M., *Arch. Entw. Mech.*, 1937, **136**, 210.

5 Gersch, M., and Ries, E., *Arch. Entw. Mech.*, 1937, **137**, 169.

6 Luther, W., *Klin. Woch.*, 1939, **18**, 682.

7 Uschin, N. P., *Arch. f. Exp. Zellforsch.*, 1931, **11**, 472.