grees of yellow pigment. Two strains were unable to ferment mannite after a period of incubation of one week, and 60 caused fermentation in intervals of less than 24 hours to 3 and 5 days. In many instances, prolonged incubation following fermentation was accompanied by an alkalinization sufficient to overcome the acidity originally formed. Of the 40 non-pathogenic strains, all Type B, and all *albus,* only 3 fermented mannite. It is interesting to note that while a slight variation from yellow pigment occurred among the Type A strains, all of the non-pathogenic strains of Type B observed remained constant in elaborating only white pigment.

The data reveal, therefore, that of a total of 102 cultures of staphylococci studied, it is possible to differentiate the immunological types A and B by fermentation of mannite within an error of 5%. This is in close agreement with the results observed by both Thompson and Khorazo[3] and S. T. Cowan of England.[5]

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Formation of Glucose-1-Phosphoric Acid in Muscle Extract.

G. T. CORI AND C. F. CORI.

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In recent experiments[1] on minced and washed frog muscle, adenylic acid, in amounts corresponding to 4 mg. P per 100 gm. muscle, increased very markedly the formation of hexosemonophosphate. The small amount of phosphorylation which occurred without addition of adenylic acid was attributed to the presence of nucleotides which had not been removed by washing, since the washed muscle contained 2 to 4 mg. % of organic P.

The first phosphorylation product was found to be glucose-1-phosphoric acid which was isolated as the crystalline brucine salt and which has since been synthesized and identified as an α-compound. This is of significance in view of the fact that glycogen, the carbohydrate from which this ester is formed, consists of α-glucosidic linkages.

Glucose-1-phosphoric acid, when added to fresh muscle extract, is converted in a few minutes to hexose-6-phosphoric acid by a wan-

[5] Personal communication.

[1] Cori, C. F., and Cori, G. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 702.

dering of the phosphate group. The same change occurs in minced and washed muscle but at a slower rate, so that after short periods of incubation and with phosphorylation accelerated by the addition of adenylic acid, the rate of formation of the 1-ester exceeds its rate of conversion to the 6-ester, these being the conditions which made possible the isolation of the new ester.

TABLE I.

Rabbit muscle extract dialyzed for 17 hours (0.01 mg. of organic P per cc.). 22 cc. extract + 12 cc. M/3 phosphate buffer pH 7 + 165 mg. glycogen + 65 mg. $MgCl_2.6H_2O$. Incubated at 24°. All determinations carried out in hexose-monophosphate fraction (= water-soluble barium salts, precipitated with alcohol). Values in mg. per 10 cc. of extract-buffer mixture.

	Without adenylic acid					With adenylic acid (0.36 mg. P per 10 cc. mixture)					
	1-ester†		6-ester*			1-ester†			6-ester*		
Time min.	hexose	P found	hexose	P found	P calc.	hexose	P found	P calc.	hexose	P found	P calc.
30	0.2	0.04	4.0	0.74	0.69	8.3	1.42	1.43	7.2	1.36	1.24
60	0.4	0.05	5.8	1.01	1.0	4.6	1.02	0.79	18.9	3.12	3.26
90	0.1	0.0	7.4	1.25	1.28	1.2	0.15	0.21	22.4	3.92	3.86

* Hexose-6-phosphoric acid, calculated from reducing power and organic P content before hydrolysis.

† Glucose-1-phosphoric acid calculated from increase in reducing power and inorganic P after 10 min. hydrolysis in N H_2SO_4.

The experiments in Table I show that a dialyzed rabbit muscle extract behaves in the same way as minced and washed frog muscle. Without addition of adenylic acid phosphorylation was slow and glucose-1-phosphoric acid did not accumulate in significant amounts. With adenylic acid added phosphorylation was increased about 4 times, resulting in an accumulation of glucose-1-phosphoric acid. Between 60 and 90 minutes phosphorylation no longer occurred and the gain in 6-ester (+ 3.5 mg.) was balanced by the decrease in 1-ester (— 3.4 mg.).

Further experiments showed that such small amounts of adenylic or inosinic acid accelerate phosphorylation that, had they been originally present in the dialyzed muscle extract, they could not have been detected by analytical methods. The smallest amount added corresponded to 0.1 mg. of adenylic acid P per 100 cc. of original extract. This amount still increased phosphorylation 3.5 times over that occurring in the extract without added adenylic acid, corresponding to a transfer of about 140 molecules of inorganic phosphate per molecule of added adenylic acid. Inosinic acid added in equivalent amounts was about one-half as active as adenylic

acid. The assumption seems justified that the muscle extract used in these experiments (and other extracts similarly prepared), since it was able to phosphorylize and convert the 1- to the 6-ester, contained minute amounts of nucleotides and of magnesium which were not removed by dialysis.

Magnesium has been shown to be an essential part of the coenzyme system of muscle.[2] In the experiments in Table II the total amount of phosphate esterified was the same without and with addition of magnesium, but the conversion of the 1- to 6-ester was much more rapid when magnesium was added. If magnesium is needed for phosphorylation, much smaller amounts are necessary than are required for the conversion of the 1- to 6-ester. When synthetic α-glucose-1-phosphoric acid was added to a dialyzed extract, addition of magnesium accelerated very markedly the transformation of this ester to the 6-ester (which was isolated as the barium salt and identified as Embden ester). At pH 5.9 in the presence of magnesium the wandering of the phosphate group was practically abolished, but the total amount of phosphate esterified was not significantly less than at pH 7.2. Heating the extract for 10 minutes at 60° caused an inactivation of the enzyme responsible for the wandering of the phosphate group.

TABLE II.

Effect of magnesium on transformation of the 1- to the 6-ester. Experimental conditions were the same as in Table I, except as noted. Values in mg. per 10 cc. extract-buffer mixture.

Adenylic (A) or inosinic (I) acid added. mg. per 10 cc. mixture	Magnesium added mg. per 10 cc. mixture	Period of incubation	1-ester as hexose	6-ester as hexose	Total esterification
A .007	0	30	8.1	1.6	9.7
A .007	2	30	0.7	7.6	8.3
A .15	0	60	12.8	4.0	16.8
A .15	2	60	1.0	15.5	16.5
I .15	0	60	5.3	3.8	9.1
I .15	2	60	0.1	9.0	9.1
0	0	60	1.4	2.7	4.1
0	2	60	0.0	3.9	3.9

Adenylic acid has been shown to act as acceptor for phosphate in organic combination, namely for the phosphate split off from phosphocreatine,[3] phosphopyruvic acid[4] or hexosediphosphoric acid.[5] The

[2] Lohmann, C., *Biochem. Z.*, 1931, **237**, 445.

[3] Lohmann, C., *Biochem. Z.*, 1934, **271**, 264.

[4] Ostern, P., Baranowski, T., and Reis, J., *Biochem. Z.*, 1935, **279**, 85.

[5] Lutwak-Mann, C., and Mann, T., *Biochem. Z.*, 1935, **281**, 140.

present experiments as well as those previously reported[1] indicate that adenylic acid can mediate still another reaction, namely, the transfer of inorganic phosphate in the formation of hexosemonophosphoric acid from glycogen. Inosinic acid is also, but less active.[6] In a dialyzed muscle extract to which adenylic or inosinic acid has been added as well as in a dialyzed extract incubated without addition of nucleotide, the first phosphorylation product is glucose-1-phosphoric acid. Addition of magnesium ions is without effect on phosphorylation but accelerates markedly the conversion of glucose-1- to hexose-6-phosphoric acid.

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Transmission of Estrogenic Substances from Animal to Animal.

ISABELLA H. PERRY AND ALLAN PALMER. (Introduced by Professor C. L. Connor.)

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We have been treating female white mice with massive doses of estrone (200 I.U.) painted on the skin.* To our astonishment a group of 25 untreated spayed mice, whose vaginal smears were all negative, all went into estrus. It had been our custom in treating or examining animals to take them from their cage, treat, and then place in a temporary box until all animals in a cage were removed, then return them to their cage. No transmission of estrogenic substances has been noted among a large number of female mice treated with subcutaneous injections of 1-10 I.U. of estrone.

To trace out the source of estrogenic stimulation we placed a standard mouse in the cage of each group receiving estrone, and one in the temporary box during a morning when the mice were being treated with estrone. A standard mouse is a young spayed adult known to respond to 2 I.U. of estrone administered subcutaneously. All of these standard mice went into estrus and remained in estrus for about 2 weeks. There could be only 2 probable routes of transmission and of reception of the estrogenic substance, *i. e.*, from the skin, and from the excreta, and through the skin, and through the

[6] Parnas, J. K., and Mochnacka, I., *Compt. rend. Soc. Biol.*, 1936, **123**, 1173.

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