and when there are no longer antibodies in the blood, reacts to a much wider range of toxin concentration. The activation energy, however, is smaller (Arrhenius coefficient 4,000–10,000)[2].

(3) The effect of toxin on volume contraction in dried muscle depends on the size of the muscle fragments: the smaller the fragments, the less pronounced is the reaction.

On the basis of these investigations we have developed a method for the detection of infectious toxins in human blood. The method is as follows. 1 gm. of frog muscle, cut into pieces 1–1·5 hr. after killing, or alternatively 0·1 gm. of dried muscle (size of fragments 1–2 mm.), is put into a glass striction vessel with a volume of about 3 ml. and with a capillary about 10 cm. long and 0·1–0·2 mm. diameter. The vessel is half filled with physiological solution saturated with air at the temperature of the experiment. The *p*H is maintained at 7·2. To this solution is added 0·1 ml. of toxic blood serum. The dried muscle is agitated with a glass rod in order to remove bubbles of air. The vessel is then filled to the top with solution and closed with a ground-glass stopper. It is then put into a water bath in which the temperature is controlled to 0·01° C. The best temperature to use for frog muscle is 29°–32° C. For dried sensitized fragments of muscle, the best temperature is 15°–20° C. The vessel is left in the bath for five minutes before readings are taken, and about one minute is needed to prepare the vessel for the experiment. Therefore there is a total interval of about six minutes before readings are taken. After this period any excess of solution at the top of the capillary is removed, so that a meniscus is formed. The position of the meniscus is then recorded every three minutes with a horizontal microscope. Readings are continued for 15–20 min. The results obtained are compared with those from a control to which no blood serum has been added. To obtain qualitative results, antitoxin is added to the blood serum. If the antitoxin corresponds to the toxin a normal contraction curve is obtained. By this technique we have[3] detected tetanus toxin in the blood and cerebrospinal fluid of sick horses, using dried muscle from rabbits immunized to tetanus. By this method, too, Galperin and Sergiev, using frog muscle, have differentiated abdominal typhoid from typhus fever by neutralizing the effect of toxin on contraction with the appropriate serum from reconvalescent blood.

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[1] Tarussof, *Bull. Exp. Biol. and Med.*, **3** (1943) (Russian).
[2] Tarussof and Hayt, *Bull. Exp. Biol. and Med.*, **9** (1944) (Russian).
[3] Tarussof and Utefskaya, *Bull. Exp. Biol. and Med.*, 12 (1944) (Russian).

A New Method of Phosphorylation

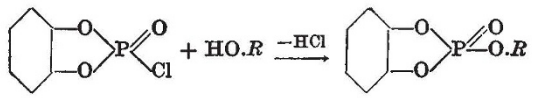
The advances in biochemistry during the past twenty years have increasingly revealed the vital part played in many biological processes by the phosphoric acid esters of sugars and amides. The synthesis *in vitro* of a number of these compounds, and of similar or related substances, would be very valuable for the study of the mechanisms of the reactions involved.

Just before the War my efforts were directed through studies on co-enzymes, to the search for a general method of phosphorylation of carbohydrates and amines, which could be applied to very sensitive glycosides. In 1942 I had to abandon work on this subject, having been engaged on war research for the Government.

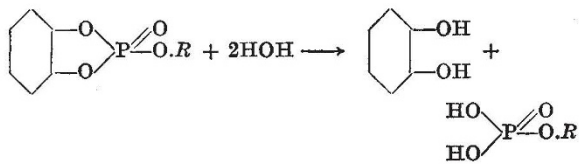
Recently, A. R. Todd and co-workers[1] published a very elegant method of phosphorylation by the use of dibenzyl chlorophosphonate as a phosphorylating agent. The reaction takes place under mild conditions; the benzyl-groups have, however, to be split up by hydrogenolysis with palladized charcoal catalyst.

The following method avoids this necessity.

Catecholoxychlorophosphine, which can be easily prepared by the action of phosphorus oxychloride on pyrocatechol, reacts readily with the hydroxyl-group in an inert solvent in presence of pyridine:



The action of water, even at low temperatures, rapidly splits up the protective catechol-group, freeing by hydrolysis the phosphoric ester:



The separation and purification of the phosphoric esters proceeds readily owing first to the good solubility of pyrocatechol in water and alcohol, and secondly to the property of phenols of not decomposing carbonates, while the phosphoric esters form metal salts of various solubilities. Further details will be published later.

This note is published by permission of the Chief Scientific Officer, Ministry of Supply, to whom my thanks are due.

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[1] Atherton, F. R., Openshaw, H. T., and Todd, A. R., *J. Chem. Soc.*, 382 (1945).

Soil Acrasieæ and their Bacterial Food Supply

Although the genera *Dictyostelium* and *Polysphondylium* were created by Brefeld[1,2] in 1869 and 1884, no study has been made in Britain of that interesting group, the Acrasieæ, to which they belong. The occurrence of *Dictyostelium* in Polish and American soils has been recorded by Krzemieniewsky[3] and by Raper and Thom[4]. *Polysphondylium* has been isolated from soil on rare occasions.

A detailed study[5,6,7,8] of the types of bacteria that are edible and inedible to soil Protozoa, especially amœbæ, has led to a method well adapted to the culture of *Dictyostelium*. It consists of spreading over the surface of plain agar (1·5 per cent washed agar containing 5 gm. per litre of sodium chloride) a few loopfuls of a suitable bacterium growing usually