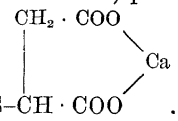
pound which, from the total yield and the proportion of gold and calcium in it, probably has the following formula:  . The theoretical yield of calcium aurothiomalate on the basis of complete precipitation is 0.9849 gm per gm of sodium aurothiomalate, and the actual yield was 0.9840 gm. The dry compound is a pale yellow powder which neither changes color nor loses weight after being heated in an oven at 100° C. for 24 hours. It is insoluble in water, alcohol and ether, but is completely soluble in tenth-normal HCl. Calcium aurothiomalate forms a uniform and relatively stable suspension in oil of sweet almonds and it was in the form of such a suspension that it was tested for toxicity and therapeutic effectiveness. While an aqueous solution of sodium aurothiomalate administered intramuscularly to 20 gm mice is lethal for 10 to 20 per cent. of animals in a dose of 6 mg, for 30 to 40 per cent. in doses of 7 to 10 mg, and for nearly 80 per cent. of animals in doses of 15 mg, it was found that all 54 mice inoculated with amounts varying from 10 to 250 mg of calcium aurothiomalate remained well over a period of 6 weeks or more without exhibiting any obvious signs of illness. The therapeutic effectiveness of calcium aurothiomalate was determined in 90 mice which were inoculated with amounts varying from 0.25 mg to 20 mg one week after the onset of arthritis. The total dose was administered intramuscularly in one injection. The arthritis disappeared completely in 90 per cent. of 70 mice which were treated with 1 mg or more of calcium aurothiomalate, but in less than 25 per cent. of those which received the 0.5 mg and 0.25 mg doses, and not at all in the 30 control mice which were untreated; 85 per cent. of 20 mice receiving the 1 mg dose made a complete recovery. Under similar conditions, *i.e.,* administering the total dose in one injection, the minimal therapeutic dose of sodium aurothiomalate is 2.0 to 2.5 mg. Calculation would show, therefore, that in mice "the margin of complete safety"[7] is at least 100 times greater for calcium aurothiomalate than for sodium aurothiomalate. A curative effect was also obtained when the total minimal effective amounts of calcium aurothiomalate were divided into ten equal doses and administered at 48-hour intervals. Calcium aurothiomalate was also found to be approximately ten times more effective therapeutically than calcium aurothioglycolate. Insoluble barium and strontium salts of aurothiomalate similar to that of calcium were also prepared, but no comparative study of their biological properties has been made as yet.

At the present time the toxicity of most available gold compounds is perhaps the greatest barrier to their more wide-spread use in the treatment of rheumatoid arthritis in man. There are also certain indications that some of the toxic manifestations in man may be due to sensitization or other factors which may not be measured by the lethal effect of a compound in mice. Only clinical trial, therefore, can indicate whether or not calcium aurothiomalate will be comparatively as safe and effective in human beings as it is in mice.

ALBERT B. SABIN
JOEL WARREN
THE CHILDREN'S HOSPITAL RESEARCH FOUNDATION AND DEPARTMENT OF PEDIATRICS
UNIVERSITY OF CINCINNATI COLLEGE OF MEDICINE

PHOSPHATE ACCEPTORS IN "RESPIRATORY PHOSPHORYLATION" IN MUSCLE TISSUE

IN our studies on the phosphorylation, coupled with the aerobic oxidation of various substrates in cardiac muscle tissue and on the action of enzyme poisons on this process, we used creatine as the acceptor of phosphate.[1] Adenylic acid proved to be a less convenient acceptor, most likely for the reason that it is deaminized. At present we have undertaken a more systematic investigation of the acceptors of phosphate. As the oxidizable substrate we used succinate, the oxidation of which to the fumarate stage is coupled with phosphate esterification, as we demonstrated some time ago.[2, 3] For the experiments cardiac muscle, red muscle and extracts from rabbit's heart have been used; the phosphate acceptors tested were glucosemonophosphate, glucose, glucose + hexokinase (from yeast), glycogen and creatine. In all experiments hexosemonophosphate displayed the most active capacity of accepting phosphate, in accordance with its behavior in brain tissue.[4] No phosphorylation takes place under anaerobic conditions; and in the absence of succinate the esterification is markedly diminished, especially upon addition of sodium fluoride. The products of hexosemonophosphate phosphorylation were shown to be hexosediphosphate (time course of hydrolysis, liberation of inorganic phosphate by phenylhydrazine) and phosphotrioses (alkali-labile phosphoric esters).

Glucose is distinctly esterified, but only in the pres-

[7] The "margin of complete safety" $\left(\frac{\text{maximal dose tolerated by nearly 100 per cent. of mice}}{\text{minimal therapeutic dose}}\right)$ is used here because it describes a property which is different from that represented by the chemotherapeutic index, $\left(\frac{\text{minimal dose lethal for 50 per cent. of animals}}{\text{minimal therapeutic dose}}\right)$.

[1] V. A. Belitzer and E. T. Tzibakova, *Biochimia* (Moscow), 4: 516, 1939.

[2] *Ibid.*

[3] Phosphorylation attending the oxidation of succinic to fumaric acid in kidney extracts has also been observed a little later and independently by S. P. Colowick, M. S. Welch and C. F. Cori, *Jour. Biol. Chem.*, 133: 359, 1940.

[4] S. Ochoa, *Nature*, 145: 747, 1940.

ence of hexokinase does it become a powerful phosphate acceptor. Glycogen induces a decrease of phosphate that is slower and less dependent on the presence of oxygen than the esterification of hexosemonophosphate. Respiratory phosphorylation in this instance is evidently involved only in the transformation into hexosediphosphate of the glucose monophosphoric ester formed by phosphorolysis. The esterification of phosphate in the presence of different acceptors is shown in Table 1.

TABLE 1

200 MG. MINCED RED MUSCLE OF RABBIT. TOTAL VOLUME 1,7 μL. SUCCINATE 0,05 M, $MgCl_2$ 0,004 μ, PHOSPHATE BUFFER 0,015 M (pH 7,4), $NaHCO_3$ 0,008 M. 40 MIN. AT 20° IN O_2. ADDITIONS: GLYCOGEN 4 MG., OTHER ACCEPTORS—25 μ M PER SAMPLE. H_3PO_4 DISAPPEARED (MG. P)

Without added acceptors	Glucose	Glucose + hexokinase	Hexose-monophosphate	Creatine	Glycogen
0,02	0,04	0,33	0,45	0,13	0,19

In spite of the presence of preformed glycogen in the muscle tissue, esterification was negligible without added phosphatic acceptor: glycogen added artificially is phosphorylated much more readily than the glycogen of the tissue. Hexosemonophosphate, likewise a constant ingredient of muscle tissue, exhibits similar behavior; a rapid accumulation of hexosediphosphate and phosphotriose in aerobiosis takes place only at the expense of added hexosemonophosphate. It is difficult at present to interpret this differential behavior of endogenous and added acceptors.

In several experiments the competition between simultaneously added acceptors was studied. It was found that when glucose + hexokinase and creatine are present together, the latter accepts almost no phosphate, and the phosphorylation of glucose predominates.

W. A. BELITZER
K. S. GOLOVSKAYA
LABORATORY OF OXIDATION PROCESSES,
DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY,
VIEM, MOSCOW, USSR

SCIENTIFIC APPARATUS AND LABORATORY METHODS

A SIMPLE METHOD FOR RECORDING ELECTROCORTICOGRAMS IN ANIMALS WITHOUT OPENING THE SKULL

THE following method enables one rapidly to implant electrodes directly in the cortex of small laboratory mammals by the simple method of thrusting Victrola needles through the skull. The needles may then be connected by wires to suitable recording or stimulating apparatus.

Common steel Victrola needles (Fig. 1a) if heated

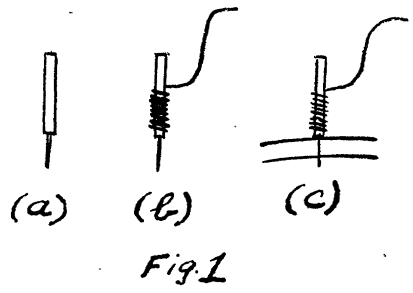


Fig. 1

red in a Bunsen flame for a few seconds lose their brittleness. The needle then is wrapped tightly with fine copper or silver wire from the lower part of its thickened shank to within about 5 mm of its top (Fig. 1b). It is well to let one end of the coiled wire extend for several inches from the shank so that clips connected to the recording apparatus can later grip the wire, thus making a flexible, non-rigid connection which will prevent pull on the implanted electrode. The needle and its wound wire is next dipped into enamel and baked, thus insulating it from subsequent contact with scalp and muscle. The point is scraped bare of enamel for a millimeter or so at the tip, and after sterilizing in alcohol the upper part of the shank is gripped firmly in a simple hand chuck by which the needle can be thrust through the skull.

The scalp and underlying muscles of the skull of the anesthetized animal are separated by a very small sterile incision 5 or 6 mm in length. The head is held firmly in one hand, the incision is spread, and the needle, gripped by the chuck, is thrust firmly through the bone by a straight downwards and rotating thrust. The enlarged shank of the needle stops the point just below the skull surface in the cortical layers (Fig. 1c). Needles thus imbedded are held firmly fast in the bone. The chuck head is carefully unscrewed so as not to loosen the needle in the bone, and the incised tissues are allowed to fall back around the projecting shank which is insulated from them by its enamel coating. A drop of collodion may be applied to the place of entry of the needle. This quickly hardens, helping to brace it and at the same time seal the slight wound from air.

Several dozen of these electrodes can be prepared in an hour and 5 or 6 of them can be implanted in almost as many minutes. When the animal recovers from anesthesia its behavior is normal and the projecting shanks appear to cause no inconvenience. Electrocorticograms recorded from them in unanesthetized rabbits, cats and rats are in all respects identical with those recorded from the exposed cortex. The