per cent. final concentration). Hemolytic units in the supernatants from the tubes which had contained active complement were estimated from the largest non-anticomplementary volume failing to show appreciable hemolysis, and were therefore actually much less than the number indicated, except in the second experiment, in which complete hemolysis was actually obtained at the level given. The results are summarized in Table 1.

TABLE 1

Precipitating system, rabbit serum	Hemolytic units of complement		Nitrogen precipitated				
	Added	Left in supernatant	Antigen, serum, saline	Antigen, serum, heat-inactivated complement	Antigen, serum, complement	Difference between active and inactivated complement	N precipitated per 1,000 hemolytic units of complement
			mg	*mg*	*mg*	*mg*	*mg*
Pn III*	1,250	<10	0.588	0.598	0.720	0.122	0.10
" "†	1,250	40	0.388	0.406	0.562	0.156	0.13
Ea-anti-Ea‡	1,000	<<75	0.478	0.484	0.604	0.120	0.12

* Antipneumococcus Type III serum, specific polysaccharide of Type III pneumococcus (S III).

† In this experiment and the one following the entire guinea-pig serum pool was filtered through Gradocol membranes of 700 mμ average pore diameter.

‡ Anti-egg albumin serum, crystalline egg albumin. In this experiment a Type I antipneumococcus horse specific precipitate, known not to fix complement (H. Zinsser and J. T. Parker, *Jour. Immunol.*, 8: 151, 1923; K. Goodner and F. L. Horsfall, Jr., *Jour. Exp. Med.*, 64: 201, 1936) was first formed in the active and inactivated complement used. Identical amounts of nitrogen (0.437, 0.437 mg per 5.0 ml) were precipitated (0.113 mg. N from the saline-SI-anti-PnI blank), but the antipneumococcus horse serum action of the horse serum used reduced the hemolytic units by one third. The value given in the table is that originally found.

It would appear, therefore, that roughly 1,000 hemolytic units, as measured above, correspond to about 0.12 mg of complement N, as defined above. If complement is actually a globulin, as now seems established,[5] this would correspond to about 0.75 mg of protein and indicate that 1 ml of guinea-pig serum ordinarily contained about 0.024 to 0.03 mg of complement N, or 0.15 to 0.20 mg of actual complement. Additional computations which follow directly from this analytical result and a discussion of some of the implications of these findings, which are being extended in several directions, will be given in a more detailed paper now in preparation.

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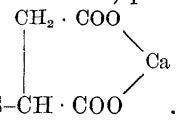
[5] T. W. B. Osborne, "Complement or Alexin," Oxford University Press, 1937; E. E. Ecker, L. Pillemer, C. B. Jones and S. Seifter, *Jour. Biol. Chem.*, 135: 347, 1940.

THE THERAPEUTIC EFFECTIVENESS OF A PRACTICALLY NONTOXIC NEW COMPOUND (CALCIUM AUROTHIOMALATE) IN EXPERIMENTAL, PROLIFERATIVE, CHRONIC ARTHRITIS OF MICE

EARLY in 1939, one of us (A. B. S.) reported that it was possible by intravenous injection of a newly discovered pleuropneumonia-like microorganism to produce an experimental, proliferative, chronic arthritis in mice which clinically and pathologically bears a close resemblance to human rheumatoid arthritis.[1] Although repeated attempts have failed to reveal the presence of such a microorganism in the human disease,[2,3,4] it was found that the experimental arthritis of mice responded to certain chemotherapeutic agents in a manner paralleling their alleged effectiveness or ineffectiveness in rheumatoid arthritis.[5] Thus, of many substances tested, the inorganic and organic gold compounds were the only ones capable of exerting a curative effect on the experimental arthritis in mice, despite the fact that they had no demonstrable action on the etiological agent *in vitro*. It was also found that the toxicity, as measured by the lethal effect on mice, and therapeutic effectiveness were a function of different properties of the gold compounds, and that depending upon their structure and mode of administration there was a wide range in the margin of safety as reflected in chemotherapeutic indexes which varied from 2 to over 30.[5] The present study obtained its orientation from the observation that while colloidal preparations of gold or of gold sulfide were therapeutically inert, a distinct, though delayed, curative effect followed the administration of large doses of an insoluble gold compound, calcium aurothioglycolate, of which mice tolerated an amount at least ten times as large as the minimal therapeutic dose.[5]

The purpose of this communication is to report that it has proved possible to prepare a compound with practically no toxicity and even greater therapeutic effectiveness simply by converting sodium aurothiomalate[6] into calcium aurothiomalate. The addition of an excess of $CaCl_2$ to a solution of sodium aurothiomalate leads to practically complete precipitation of a com-

[1] A. B. Sabin, SCIENCE, 89: 228, 1939.
[2] *Ibid.*, SCIENCE, 90: 18, 1939.
[3] A. B. Sabin and B. Johnson, *Proc. Soc. Exp. Biol. and Med.*, 44: 565, 1940.
[4] G. M. Findlay, R. D. Mackenzie and F. O. MacCallum, *Brit. Jour. Exp. Path.*, 21: 13, 1940.
[5] A. B. Sabin and J. Warren, *Jour. Bact.*, 40: 823, 1940 (in press); also unpublished observations.
[6] We are grateful to Merck and Company for supplying the sodium aurothiomalate (myochrysine) used in this investigation.

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.

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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.