THE CURATIVE EFFECT OF CERTAIN GOLD COMPOUNDS ON EXPERIMENTAL, PROLIFERATIVE, CHRONIC ARTHRITIS IN MICE[1]

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The purpose of this communication is to report experiments which demonstrate (1) the curative effect of certain gold compounds on an experimental, proliferative, and progressive, chronic arthritis in mice, (2) the conditions under which these compounds exert their optimum effect, (3) the relation between chemical composition and solubility on one hand and toxicity and therapeutic effectiveness on the other, and (4) the absence of any apparent *in vitro* effect of a therapeutically active compound, or of the blood of mice treated with it, against the microbial agent which causes the arthritis.

The experimental disease which has lent itself to the investigation of these questions has already been described briefly (Sabin, 1939a), and our present understanding of it and of the etiological microbial agent is somewhat as follows. Many different stocks of mice have been found to be carriers of a number of biologically and immunologically different types of filtrable microörganisms of the pleuropneumonia group (Sabin, 1939b). These microorganisms have been found in normal mice chiefly in the mucosa of the nose and sinuses and on the conjunctiva, and, only occasionally and in small numbers, in the lungs and brain; but examination of thousands of mice has thus far failed to reveal any spontaneous arthritis. However, when some of these normal microbial inhabitants of mice are cultured *in vitro* and injected

[1] Part of the work presented in this communication was carried out while one of us (A. B. S.) was on the staff of the Rockefeller Institute for Medical Research.

intravenously even into mice of the same stock, they give rise to a progressive, proliferative chronic arthritis. The studies carried out thus far by one of us (A. B. S.) indicate that the pleuropneumonia-like microörganisms of mice have distinct tissue affinities (chiefly for those of mesenchymal origin) and in the animal body multiply within susceptible cells, even though outside the body it is possible to cultivate them in the absence of living cells. Some strains have been found to attack more types of mesenchymal cells than others, and the one selected for the present study (a type B strain) has its chief affinity for the joints. After a single intravenous injection of this strain the blood and organs are as a rule sterile within 24 hours and remain so while the arthritis develops and positive cultures are obtained from the joint tissues. Following a single intravenous injection of 0.5 ml. of a properly growing 24- to 48-hour culture of this strain (72-hour cultures are not pathogenic) 90 to 100 per cent of mice develop a polyarthritis which is migratory at first and finally progressive and chronic, leading to ankylosis in many instances between 2 to 4 months after inoculation. The incidence of ankylosis was very high (over 70 per cent) with this strain early after isolation, and ankylosis, as well as the essentially chronic character of the arthritis, have diminished with cultures between the 50th and 75th passage.

A Giemsa-stained film of a 48-hour serum glucose broth culture, used for the production of the experimental arthritis, is shown in figure 1. The complex morphology of this microbial agent is one of its striking characteristics but it is necessary to point out that the minimal reproductive unit is an elementary-body-like structure which according to gradocol membrane measurements is of the same order of magnitude as vaccinia virus. The gross appearance of the arthritis is illustrated in figure 2. The anterior and posterior extremities are equally affected and symmetrical involvement is quite frequent. Fusiform swelling of the digits is very common. The knee-joints are also affected but their involvement is not readily discernible clinically until partial or complete ankylosis has set in. Pathological changes are found in the capsule, synovia, cartilage and subchondral

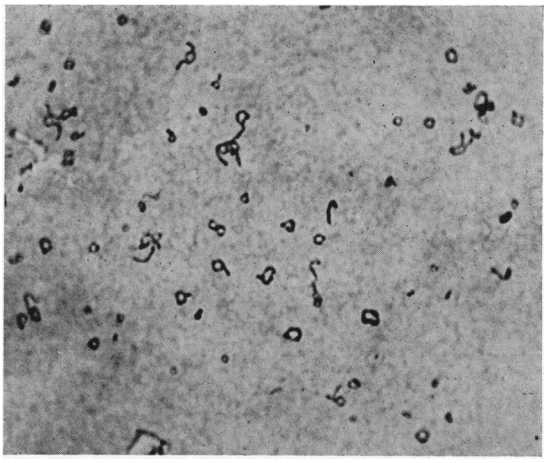


FIG. 1. FILM OF 48-HOUR CULTURE OF THE TYPE B MICROORGANISM USED FOR THE PRODUCTION OF EXPERIMENTAL CHRONIC ARTHRITIS
Giemsa, × 1,000

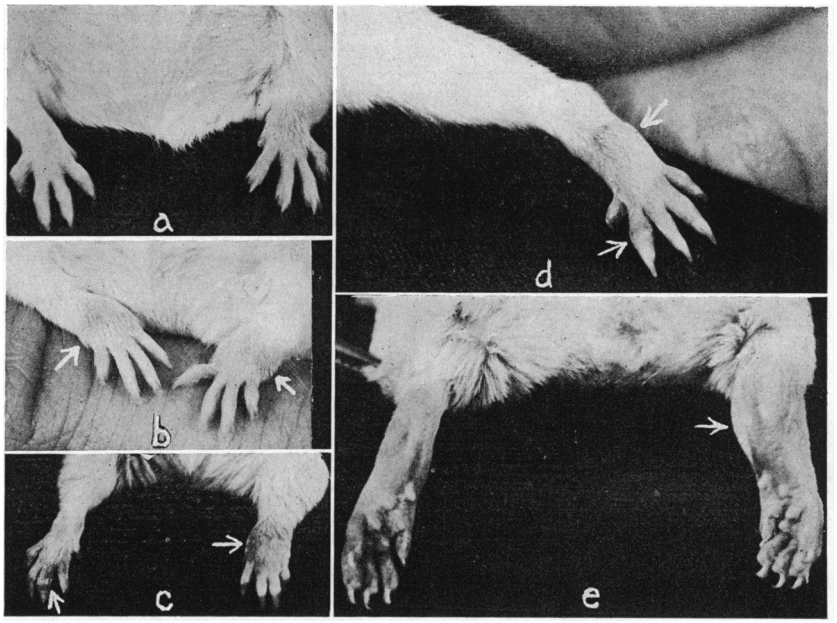


FIG. 2. GROSS APPEARANCE OF AFFECTED JOINTS
a, Anterior extremities of normal mouse. *b*, *c*, *d*, and *e*, extremities of mice with experimental arthritis of 1 to 2 weeks' duration; arrows point to involved joints. × 1.6.

bone-marrow (Sabin, 1940). The essentially proliferative character of the process is apparent in the synovia as early as the second day after swelling of the joint is discernible and becomes more marked subsequently, affecting the capsule, synovia and

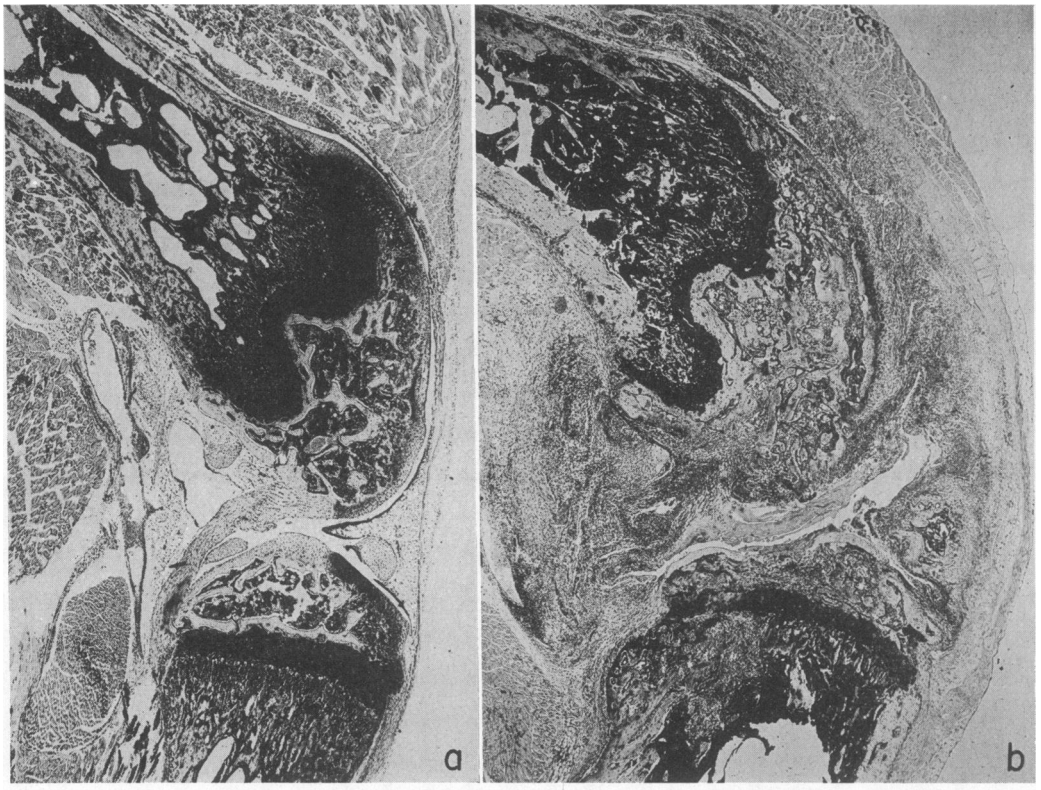


FIG. 3. *a*, KNEE OF NORMAL MOUSE TO BE COMPARED WITH *b*, KNEE OF AFFECTED MOUSE 33 DAYS AFTER INJECTION OF CULTURE

Note (1) proliferation of tissue affecting synovia and capsule, articulating cartilage, and subchondral bone-marrow; (2) obliteration of free joint space by proliferated tissue; and (3) necrosis of articulating cartilage of femur and tibia. × 19.

perichondrium to such an extent that obliteration of the free joint space may result from an overgrowth of the proliferating tissue (see knee-joint in fig. 3). Actual destruction of cartilage was not seen until about the 4th week, and at 7 weeks one may find (as in the wrist in fig. 4) that the normal articulating cartilage

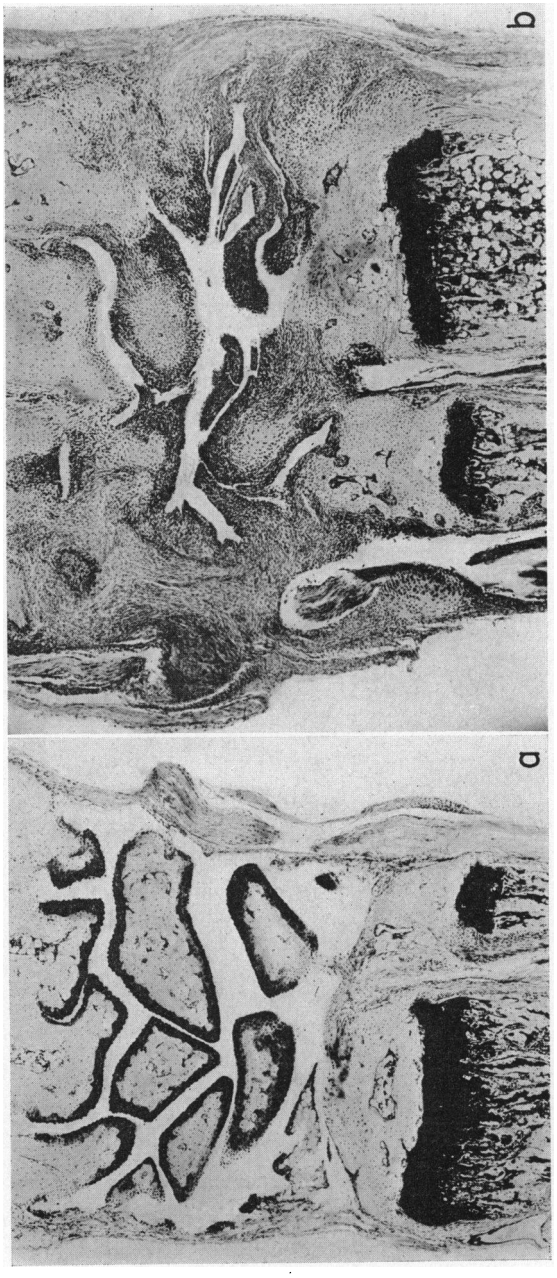


FIG. 4. *a*, WRIST OF NORMAL MOUSE FOR COMPARISON WITH *b*, WRIST OF MOUSE WITH EXPERIMENTAL ARTHRITIS 50 DAYS AFTER SINGLE INTRAVENOUS INJECTION OF CULTURE

Note (1) disappearance of most of the articulating cartilage and replacement by proliferating undifferentiated cells which may be immature chondroblasts or osteoblasts; (2) proliferative reaction in synovia and capsule. × 34.

has been replaced by undifferentiated cells which may be immature chondroblasts or osteoblasts. In a completely ankylosed joint, at about 5 months, one finds that considerable ossification has occurred in the distorted articulating surfaces which are joined by dense fibrous tissue.

While the gross appearance, the clinical course and the pathology of this experimental disease in mice are in many respects similar to rheumatoid arthritis in man, repeated attempts to obtain a similar microörganism from exudates and tissues of the human disease have been unsuccessful (Sabin, 1939b). Nevertheless, it appeared possible that certain problems relating to the therapy of human rheumatoid arthritis might be investigated with the help of this experimental disease in mice. All sorts of chemical compounds and biological agents have been tried empirically in rheumatoid arthritis, but because of the nature of the disease evaluation of results is so difficult that the editorial committee of the American Rheumatism Association was prompted to make the following comment in the Fifth Rheumatism Review (1939) (Hench *et al.*, 1939): "The curve of acceptance of most "new" treatments for arthritis that are destined to be discarded, rises rather rapidly, reaches its peak in about three to five years, then falls as adverse reports begin to outnumber the optimistic ones." Since Forestier's paper in 1929 there have been numerous communications especially from France and England, and lately from American investigators reporting with varying degrees of enthusiasm on the benficial effects of various gold compounds in rheumatoid arthritis, and the Fifth Rheumatism Review referred to this subject as follows: "American physicians, aware of the supposed value of gold for arthritis but concerned about its toxicity, have been slow to use it, expecting it would die out or be made safer. It therefore seems significant that the curve of acceptance of chrysotherapy is still rising after 10 years of use." It was of interest, therefore, to determine first whether compounds which have been reported to have a beneficial effect on the human disease, would behave similarly with regard to the experimental mouse arthritis. Quite recently Collier (1939) working with a spontaneous rat "ar-

thritis," transmissible by periarticular injection of infected tissues, and Findlay, Mackenzie and MacCallum (1940), with a spontaneous rat arthritis caused by a pleuropneumonia-like microörganism and some other pyogenic pleuropneumonia-like microörganisms of the rat, demonstrated that a number of gold compounds administered at the time of infection and subsequently could prevent the development of "arthritis" in most of the treated animals. With the problems of human therapy as a basis, our own experiments were curative rather than prophylactic in that treatment was not begun until the arthritis was well developed.

METHOD OF INVESTIGATION AND EVALUATION OF RESULTS

Culture. In order to be able to produce arthritis in at least 90 per cent of mice, it is necessary to have a culture that is growing under optimum conditions. The culture medium is important, in that a final pH that is more acid than 7.6 to 7.8 or the use of commercially dried beef heart instead of the fresh tissue for the preparation of the infusion has yielded no growth or poor growth, which upon intravenous injection into mice produced no arthritis. The culture medium we have used successfully is made up as follows: The broth is a fresh beef-heart infusion, containing 1 per cent peptone, and has a pH of 7.8 to 8.0 after sterilization. Glucose is added to a concentration of 0.5 per cent and bovine serum or ascitic fluid, which has been freshly filtered through a single Seitz disc, to a concentration of 10 to 30 per cent.

In order to overcome the gradual modification which occurs with prolonged *in vitro* passage, no cultures were used beyond the 50th transfer. Cultures in the 37th and the 43rd transfer were dried from the frozen state according to Swift's technique for preserving bacteria (1937). Since growth from the dried specimens is slow and not optimum, two or three rapid passages were made prior to animal inoculation. An actively growing culture would also be stored in the refrigerator and transfers from it could be made as required during the course of about a month. The inoculum for a culture that is to be used for mouse inoculation is obtained from a tube in which growth appeared in 24 to

48 hours and the amount is 1 or 2 parts of actively growing culture in 50 parts of fresh medium.

Animals. Albino mice, 3 to 4 weeks of age, were used in all experiments. 0.5 ml. of the culture was injected intravenously.

Observations and records. The minimum number of mice injected at one time was 30, but the usual number in a single experiment varied from 50 to 120. Mice were caged in groups of 10; each one was numbered so that a record could be kept of each mouse over a period of months. The joints of the anterior and posterior extremities were observed at frequent intervals, and an "arthrogram" (a diagrammatic record of the affected joints) of the type shown in chart I was made. With a good culture, arthritis often appeared as early as the 3rd or 4th day.

Therapy. No compound was administered until about a week after the appearance of arthritis in the majority of mice, which by that time were 5 to 6 weeks old and weighed 18 to 22 grams. The single minimal lethal dose for approximately 20-gram mice was determined for most compounds before they were administered. Most compounds that could be injected intravenously were given by that route, although the effects of administration by the intramuscular and subcutaneous routes were also studied. In each experiment there was a group of at least 10 mice which were untreated and which served as controls for the particular culture that was injected, as well as groups of animals given therapeutically ineffective doses or compounds, so that mice in which a definite therapeutic effect was observed would stand out in contrast to the others.

THERAPEUTIC EFFECT OF VARIOUS GOLD COMPOUNDS

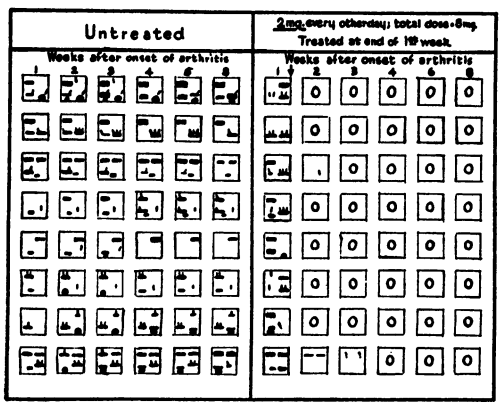
A preliminary experiment, in which a group of 10 mice was given 2 mgm. of sodium aurothiomalate (myochrysine) intravenously every other day one week after the onset of arthritis clearly demonstrated the remarkable therapeutic effect of this compound. Within 4 days there was a distinct diminution in the swelling of all the affected joints and by the end of the first week the arthritis had disappeared completely in 80 per cent of the mice. With this observation at hand, a systematic study

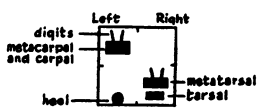
was undertaken to determine the influence of dosage, route of administration, effect of optimum doses at various stages of the disease, and the relation between the chemical composition of various gold compounds and their therapeutic and toxic effects. The compounds used in the treatment of human rheumatoid arthritis have been inorganic, organic and colloidal. As an example of an inorganic compound the commonly used double thiosulfate of gold and sodium (sanocrysin) was selected; myochrysine, triphal, and solganal were the soluble organic compounds, and calaurol or myoral (calcium aurothioglycolate) was used as an example of an insoluble organic compound suspended in oil; two colloidal preparations, aurol-sulfide and 1 per cent metallic colloidal gold, were tested because they had been used in human beings in an attempt to avoid the toxic reactions which occur with the soluble compounds.[2] Because all the organic compounds contain sulfur, which is necessary to bind the gold to the organic radical, and because sanocrysin also contains sulfur, although in a different linkage, it was desirable to test gold chloride to determine whether or not gold itself could exert a therapeutic effect. The amount of compound that could bring about the complete disappearance of arthritis in 50 per cent or more of mice within the two-month period of observation was taken as the minimal therapeutic dose for comparative purposes.

Effect of sodium aurothiomalate (myochrysine): (a) *Influence of dosage.* To determine the minimal therapeutic dose, as well as to observe the relation between various doses of myochrysine and the rate of complete disappearance of arthritis, different groups of mice were given 2, 1, 0.5, 0.25, 0.1, and 0.02 mgm. of the compound intravenously approximately 1 week after the first appearance of arthritis. Each dose was repeated every 48 hours until the arthritis disappeared completely in at least 50 per cent of the mice or until 10 doses had been administered. While there is at

[2] We are grateful to Merck and Company for supplying the myochrysine used in these experiments, to Dr. C. W. Jungeblut for the solganal, to the Hille Laboratories of Chicago for the aurol-sulfide, and to Crookes Laboratories of New York for the colloidal gold preparation.

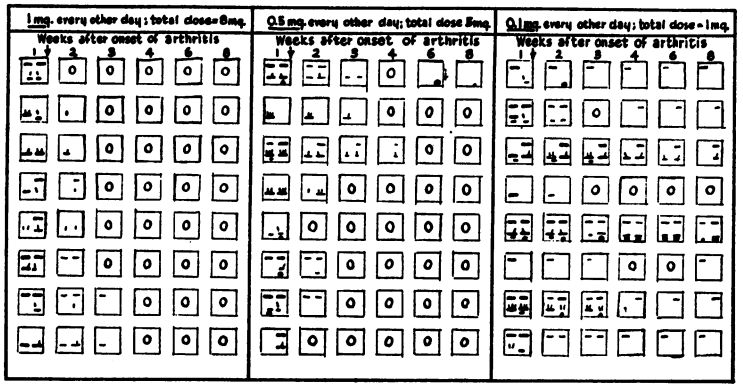
Effect of Sodium Aurothiomalate (Myochrysine) on Experimental Proliferative Arthritis





EACH SQUARE REPRESENTS A MOUSE, AND THE THICKNESS OF THE SYMBOLS INDICATES THE SEVERITY OF ARTHRITIS

EFFECT OF DIFFERENT DOSES — THERAPY STARTED AT END OF FIRST WEEK.



EFFECT OF STARTING THERAPY AT DIFFERENT STAGES OF DISEASE; DOSE = 2 mg. EVERY OTHER DAY

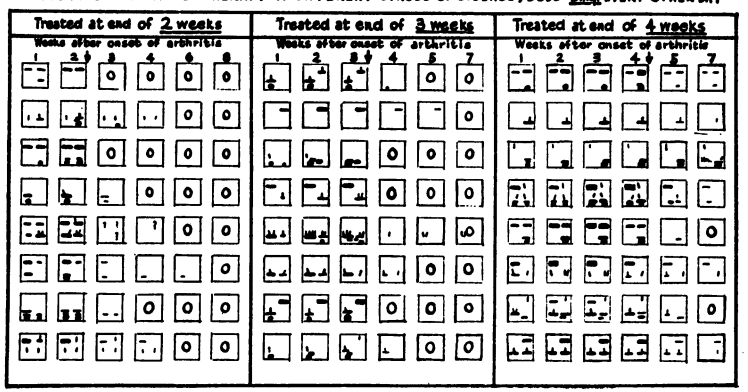


CHART I

first a diminution in the severity of involvement, both as regards degree of swelling and number of joints affected in each mouse, it appeared desirable to use complete disappearance of the arthritis in an animal as the end-point for quantitative estimation. Arthrograms of groups of 8 mice, which were either untreated or had received varying amounts of myochrysine, are presented in chart I, which is intended to show the qualitative changes which occur in individual mice over a period of 8 weeks, while chart II presents the quantitative aspects of the same experiment.

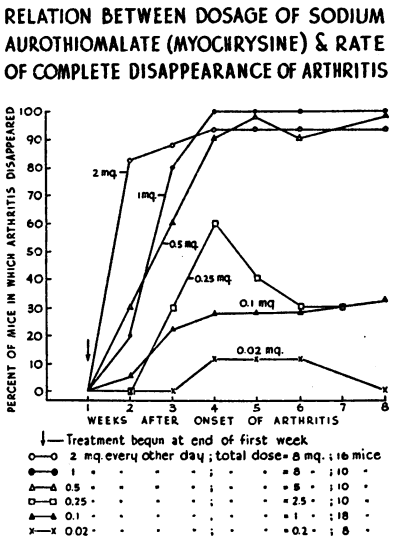


CHART II

The best result was obtained with the 2 mgm. dose since the arthritis disappeared in 82 per cent of the mice within the first week of treatment, in 88 per cent by the end of the 2nd, and in 94 per cent at the end of the 3rd week. While ultimately there was a clearing in all mice, there was a recurrence of the arthritis in a single digit of one animal during the 4th week, so that the final record remained at 94 per cent. With the 1 mgm. dose it was not until the end of the 2nd week that the arthritis had completely disappeared in 80 per cent of the mice, although at the

end of the first week there was already a halt to the further progress of the disease and a definite diminution in the severity and number of involved joints; 3 weeks after the beginning of therapy (or 4 weeks after the onset) all mice were free of arthritis. With the 0.5 mgm. dose the rate of complete disappearance of arthritis was still slower, although by the 4th week all had cleared; but there was a recurrence in a single mouse which disappeared, however, after further therapy (see chart I). The test with the 0.25 mgm. dose was not carried out until it was found that both the 0.02 and 0.1 mgm. amounts were definitely below the requirements of the minimal therapeutic dose. While the rate of disappearance of arthritis was slow, the 0.25 mgm. dose was regarded as the minimal therapeutic dose since arthritis completely disappeared in 60 per cent of the mice. It should be noted, however, that after cessation of treatment the incidence of recurrence was high, and it is open to question whether such a dose should be considered as the minimal therapeutic amount. The mice treated with 0.02 mgm. progressed as did the untreated animals, while among those treated with 0.1 mgm. there were quite a number in which the arthritis was much less severe or had disappeared altogether and then reappeared after a period of several weeks.

It is clear from these tests that not only the rate of disappearance of arthritis but also the percentage of animals in which it disappears is determined by the amount of myochrysine that is administered, and that on the borderline of the effective dose there is a high incidence of recurrence after the cessation of treatment. The mice which received individual doses higher than 0.25 mgm. and were free of arthritis at 6 to 8 weeks were still free when last observed at 6 months. That a single dose, provided it is large enough, is capable of exerting a curative effect was evident in a test in which the arthritis completely disappeared in 5 of 7 mice which were given 2 mgm. of myochrysine intravenously in one injection, and it is interesting to note that it took 2 to 3 weeks for the effect to occur. When it is recalled that repetition of the 2 mgm. dose every 48 hours brings about a similar therapeutic effect within 5 to 7 days after the

beginning of therapy, it becomes clear how dosage affects the outcome. Furthermore, when the same total dose (8 mgm.) was given over a period of 2 weeks in 1 mgm. amounts on alternate days it took twice as long for an equivalent therapeutic effect to occur as when administered over a period of 1 week in 2 mgm. amounts.

(b) *Therapeutic effect in relation to stage of disease when treatment is begun.* The question considered in the next series of tests was whether a compound like myochrysine, which exerted an excellent therapeutic effect when administered one week after

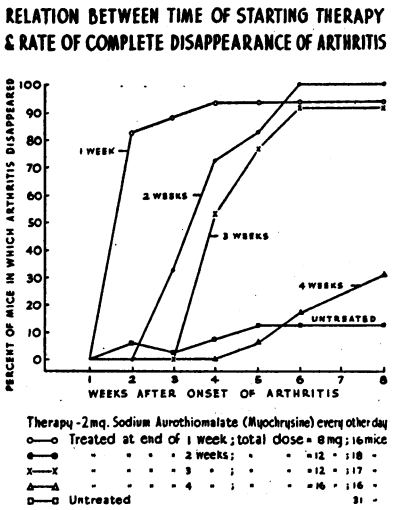
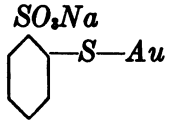


CHART III

the onset of arthritis, could produce similar effects at all stages of the disease, or whether there was some limiting time beyond which it would become inactive. Charts I and III show the effect of beginning therapy 2, 3, and 4 weeks after the onset of arthritis. Two milligrams of myochrysine was given intravenously every other day for a period of 2 to 3 weeks. It may be seen that when therapy was delayed as long as 3 weeks, a good result was still attainable, although the rate of complete disappearance of arthritis was considerably slower, as compared with the group which was treated one week after onset. When

treatment was delayed as long as 4 weeks, it was evident that in the majority of mice some joints, if not all, had progressed to a point where this therapy was no longer effective. The same was found to be true in a small group of mice which were treated similarly 10 weeks after the onset of arthritis, i.e. while the arthritis disappeared in some joints, there were others in the same mouse in which the condition was unaffected. Mice with ankylosis, 5 and 6 months after onset, were not visibly affected by treatment, although again there was possibly a good effect on an occasional joint which was not ankylosed. The time when this therapy can no longer bring about a complete disappearance of arthritis coincides with the stage when distinct cartilage destruction is first observed, and it would appear that a return to normal can be expected only in joints with cartilage that is not too markedly involved by the disease process.

Relation between route of inoculation and effect. Several groups of mice were given myochrysine intramuscularly or subcutaneously one week after onset of arthritis. With the 2 mgm. dose either intramuscularly (17 mice) or subcutaneously (8 mice) the therapeutic result was both as good and as rapid as after intravenous administration of the drug. By the intramuscular route, 0.5 mgm. was effective and the 0.1 mgm. dose was not, indicating that the intramuscular and intravenous minimal therpeutic doses were probably in the same range.



Effect of solganal—$NH \cdot CH_2SO_3Na$. Solganal is an aurothio derivative of the formaldehyde bisulfite addition product of sodium sulfanilate and is not to be confused with solganol B which is aurothioglucose. Only enough solganal was available to treat eight mice which were given 2.75 mgm. (i.e. 1 mgm. gold) on alternate days for 4 doses. The response was as rapid and complete as with the equivalent dosage of myochrysine, the arthritis having disappeared in all the mice without recurrence for at least 6 months.

Effect of triphal (*sodium aurothiobenzimid-azole-carboxylic acid*). This compound is still under investigation but in view of the fact that it was found to be considerably more toxic by the intravenous route than myochrysine, it should be noted that the available results indicate that, while it can also exert a curative effect, the minimal therapeutic dose does not appear to be smaller than that of myochrysine.

Effect of sanocrysin (*double thiosulfate of gold and sodium*). The inorganic compound, sanocrysin, as will be shown later, is

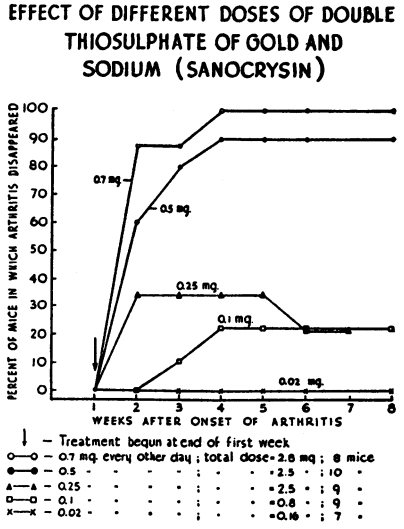


CHART IV

definitely more toxic than myochrysine, and its therapeutic effect had to be tested in smaller doses. The drug was injected intravenously one week after the onset of arthritis and the results shown in chart IV indicate that it can be quite as effective as myochrysine. While the minimal therapeutic dose in milligrams of sanocrysin (37 per cent gold) is greater than that of myochrysine (50 per cent gold), it is not improbable that in terms of actual gold, the minimal effective amount may be the same for both compounds.

Effect of calcium aurothioglycolate (*calaurol, myoral*). Calaurol,

an insoluble, organic gold compound, containing 64 per cent gold, was available as a 10 per cent suspension in oil of sweet almonds and was injected intramuscularly in 10 mgm. doses which were repeated every other day until 80 mgm. were administered. The drug was given to 9 mice one week after the onset of arthritis. For two weeks after the beginning of therapy there was no noticeable effect, but during the 3rd week the arthritis began to diminish and disappear so that by the end of that week it had completely disappeared in 7 of the 9 mice; in the course of the next two weeks the slight residual involvement of two joints of the remaining two mice also cleared up, and there was no recurrence in any of the animals. The therapeutic effect exerted by this "insoluble" organic gold compound indicates that it is probably slightly soluble in the body and is slowly absorbed from the site of inoculation. The fact that the largest amount of it (100 mgm. in 1 ml. of oil) that was given as a single dose to 20-gram mice was not toxic, suggests that further, more detailed, investigation of this type of compound is definitely indicated.

Effect of colloidal gold. Seventeen mice were treated with Aurol-Sulfide, which is described as a 0.5 per cent aqueous colloidal gold sulfide preparation containing 87 per cent gold and 13 per cent sulfur. One-half milliliter, containing 2.5 mgm. of the compound, which is the largest amount of the preparation that could be given in a single intravenous injection, to 20-gram mice, gave rise to severe tremors and prostration immediately after administration but all animals recovered and remained well. The first group of 9 mice received 1 mgm. every other day until 8 mgm. were administered; the first six doses were given intravenously but the remaining ones had to be injected intramuscularly because the veins had become occluded. Another group of 8 mice received a series of 9 doses of 1.5 mgm. each in the same manner. Of the 17 mice, 5 died during the first three weeks of treatment, and in the remaining 12 there was no therapeutic effect whatever. Although the affected extremities became colored in a manner that suggested a concentrated deposition of the colloid in the inflamed joints, the arthritis became progres-

sively more severe in the treated mice, and there was no difference between them and the untreated animals except that none of the untreated ones died.

Another colloidal preparation which was described as a 1 per cent suspension of metallic gold protected against precipitation by a small amount of gelatin and other "emulsoid colloids," was given to 9 mice, one week after the onset of arthritis. Five milligrams of colloidal gold were injected intramuscularly every 48 hours, but the injections were stopped after 20 mgm. had been administered because 3 of the 9 mice had died. The 6 surviving ones showed deposition of the colloid in the affected joints, but there was again no therapeutic effect of any kind.

Is sulfur a necessary part of a therapeutically effective gold compound? Effect of gold chloride. The following tests were carried out chiefly to determine whether a gold compound without any sulfur in it could exert a therapeutic effect on the experimental mouse arthritis. Gold chloride, $AuCl_3 \cdot HCl \cdot 3H_2O$, is highly toxic on intravenous administration; while the single minimal lethal dose (killing 50 per cent or more of 20-gram mice) is slightly more than 0.5 mgm., 0.1 mgm. was selected as the dose for treatment, because even 0.2 mgm. was found to kill a varying percentage of mice. Eight or nine doses, over a period of 2 to 3 weeks, were administered to 18 mice, intravenously at first and then intramuscularly as the veins became occluded after 3 or 4 injections. None of these animals died and they behaved in general like the ones treated with 0.1 mgm. of myochrysine, except that 5 weeks after the beginning of therapy the arthritis had completely disappeared in 50 per cent of the gold chloride group. Whether or not larger amounts of gold chloride could exert a greater and more rapid therapeutic effect could be determined only by the administration of doses which would kill a certain number of the mice. It was found, for example, that mice would survive a single subcutaneous injection of 15 mgm. of gold chloride although there was extensive local necrosis; while repeated injections of 2 mgm. amounts every other day killed about 50 per cent of the mice in one to 2 weeks. These necrotizing and toxic properties of gold chloride on subcutaneous in-

jection were not appreciably modified by adjusting the solution to a pH of 7.0 with tenth-normal sodium hydroxide, even though this neutral preparation was less toxic on intravenous injection. Twenty mice received 2 mgm. amounts of the acid or neutral solution of gold chloride subcutaneously one week after the onset of arthritis; 11 mice had a total dose of 7 mgm. of the acid gold chloride and 9 mice a total dose of 14 mgm. of the neutral solution. Of the 20 mice, 7 died within less than a week, before any evidence of a therapeutic effect could be seen; in the remaining 13, the arthritis disappeared completely between the first and second

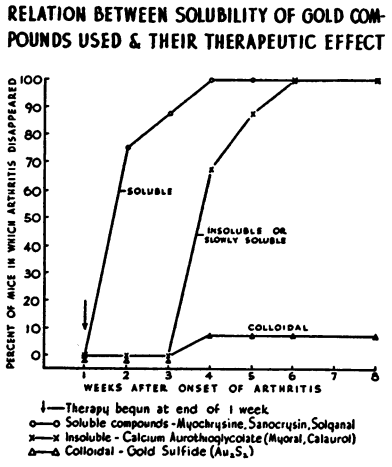


CHART V

weeks after the beginning of therapy; 4 additional mice died during that period, but the 9 survivors remained free of arthritis.

Thus, while gold chloride is too toxic for practical and even experimental therapeutic purposes, it is nevertheless clear that a gold compound without sulfur in the molecule is also capable of exerting a curative effect on the experimental mouse arthritis.

Solubility and therapeutic effect. The foregoing experiments suggest that the therapeutic effect exhibited by the gold compounds studied is dependent upon the availability of a certain concentration of chemically reactive gold, probably in an ionic state. This relationship is illustrated graphically in chart V.

The greatest and most rapid therapeutic effect was obtained with the largest doses of the soluble, crystalloid, inorganic or organic compounds; with an "insoluble" or slowly soluble compound from which chemically active gold is probably slowly liberated, the therapeutic effect is markedly delayed but nevertheless striking. In the colloidal state, however, gold has no therapeutic properties.

THE INEFFECTIVENESS OF SOME OTHER CHEMOTHERAPEUTIC AGENTS

Sodium salicylate. Sodium salicylate had no effect when administered subcutaneously either before or after the appearance of arthritis. Twenty mice were used in this test, each one receiving a total dose of 40 mgm. over a period of 8 days.

Bismuth subsalicylate. The intramuscular M.L.D. of this compound for 20-gram mice was between 40 and 60 mgm. Each of 9 mice was given 2.6 mgm. on alternate days for 8 doses (total—20.8 mgm.) and 10 mice received 13 mgm. each, every other day for 10 doses (total—130 mgm.) one week after the onset of arthritis; there was no evidence of a curative effect in either group.

Sodium sulfapyridine. The single intravenous M.L.D. of this compound for 20-gram mice was 10 to 12 mgm. Each of 10 mice received 5 mgm. intravenously every other day until a total dose of 35.0 mgm. had been administered. Treatment was started one week after the onset of arthritis and there was no evidence of any therapeutic effect.

Neoarsphenamine. The intravenous M.L.D. for 20-gram mice was 8 mgm. Administered intravenously every other day in 5 mgm. amounts to each of 10 mice for 8 doses, the drug exerted no appreciable effect.

SUMMARY OF CHEMOTHERAPEUTIC EXPERIMENTS

The essential results of all the preceding experiments, summarized in table 1, show that arthritis disappeared completely in 96 per cent of 171 mice which were treated with suitable gold compounds in adequate dosage and at the proper time, but in

only 7 per cent of 70 mice which were untreated and in only 5 per cent of 77 mice which received colloidal gold, sodium salicylate, bismuth subsalicylate, neoarsphenamine or sodium sulfapyridine in simultaneous tests and under identical conditions.

TOXICITY OF THERAPEUTICALLY EFFECTIVE COMPOUNDS

If the same factor or factors were responsible both for the therapeutic effectiveness and the toxicity of a compound one would expect the chemotherapeutic indexes of various active preparations to be of the same order of magnitude, and there would be little or no basis for attempting to find or synthesize

TABLE 1

Results of chemotherapeutic experiments

THERAPY	NUMBER OF MICE	PERCENTAGE OF MICE IN WHICH ARTHRITIS COMPLETELY DISAPPEARED WITHIN 8 WEEKS
Suitable gold compounds in adequate dosage at proper time	171	96 per cent
Suitable gold compounds, but in inadequate dosage or too late in disease	83	0 to 33 per cent in different groups
Colloidal gold, sodium salicylate, bismuth, subsalicylate, sodium sulfapyridine, neoarsphenamine	77	5 per cent
Untreated	70	7 per cent

additional gold compounds with a still greater margin of safety between the curative and toxic dosages. The toxicity of a number of gold compounds of different chemical composition was, therefore, investigated in some detail and the results showed that there can be a wide range in the toxicity as well as in the chemotherapeutic index $\left(\frac{\text{minimal lethal dose}}{\text{minimal therapeutic dose}}\right)$ of the different compounds.

The single minimal lethal dose (M.L.D.) was determined in at least two steps. In a preliminary test, groups of two to four 20-gram mice were injected with a number of graded doses in

order to find the zone which separated death and survival. The test was then repeated with a minimum of 6 mice for each dose within that zone, and the amount that killed 50 per cent or more of 20-gram mice during an observation period of 2 to 3 weeks was considered as the single M.L.D. The results of such tests are shown in table 2. For the intravenous route the single M.L.D. of a number of therapeutically effective gold compounds was found to be approximately as follows:[3]

Gold chloride, acid	0.75 mgm.	or 0.37 mgm. gold
Gold chloride, neutral	2.0 mgm.	or 1.0 mgm. gold
Double thiosulphate of gold and sodium (sanocrysin)	1.0 mgm.	or 0.37 mgm. gold
Sodium auro-thio-benzimid-azole-carboxylic acid (triphal)	2.0 mgm.	or 0.88 mgm. gold
Sodium aurothiomalate (myochrysine)	8.0 mgm.	or 4.00 mgm. gold

It is thus clear that the amount of gold in an M.L.D. can vary over a wide range in both inorganic and organic compounds. Since gold is the constituent responsible for the toxicity of all these soluble compounds, it would appear that the nature of the rest of the molecule and the manner in which it is combined with or dissociated from it probably determine not only how much of a certain substance shall be lethal but also (because the toxic signs after intravenous injection are not the same with all compounds) the type of toxicity that is produced. Myochrysine, for example, invariably killed with signs suggestive of respiratory failure within a few seconds after the injection and the unaffected mice, with only two exceptions, remained well thereafter. With sanocrysin, on the other hand, death was always delayed; and while 20 times the M.L.D. gave rise to convulsions and death in 6 to 9 minutes, smaller amounts frequently produced no obvious signs for 2 to 3 days with a still longer delay before death occurred. The sick sanocrysin mice showed particularly severe

[3] Furthur experience with toxicity tests has convinced us that for an accurate determination of the MLD approximately 20 mice must be used for each dose in the critical zone. Using these larger numbers of mice the intravenous MLD was found to be 7.5 mgm. instead of 8 mgm. for myochrysine, and 3 mgm. instead of 2 mgm. for triphal.

nervous signs consisting of marked gross tremors and ataxic gait associated with ruffled fur.

The relative behavior of these compounds after intramuscular injection was also peculiar in several respects. Myochrysine, even with the largest doses, killed after a delay of one or more days; the sick mice showed a hunched back, rapid respiration, and while they were hypersensitive to stimuli they were abnormally quiet when undisturbed and had none of the nervous signs exhibited by the sanocrysin animals and, furthermore, many of the sick mice recovered. While there was considerable irregularity in individual behavior, so that the exact intramuscular M.L.D. for myochrysine could not be interpolated from the number of mice that were used, it is nevertheless obvious that more of the compound can be tolerated by the intramuscular than by the intravenous route. There was greater regularity in reaction after subcutaneous injection and it appears that myochrysine is only about one half as toxic subcutaneously as intravenously.[4] This disproportion in the M.L.D. by different routes was even more marked for some of the other compounds. Gold chloride injected subcutaneously could be tolerated in a dose that was at least 20 times the intravenous M.L.D.; the extensive necrosis and gangrene which developed in a few days suggested that it practically all combined with the tissues at the site of inoculation. Gold chloride adjusted to pH 7.0 still produced local necrosis. On the other hand another inorganic compound, sanocrysin, which can be injected intramuscularly without producing appreciable local reaction was much more toxic and the intramuscular M.L.D. was only twice as large as the intravenous M.L.D.[5] That a disproportionate difference in toxicity by different routes can also occur with an organic compound containing the Au-S linkage is evident from the tests with triphal, which was approximately 3 times more toxic by the intravenous

[4] Additional tests with larger numbers of mice indicated that the MLD of myochrysine is 15 mgm. by the intramuscular route and 18 mgm. by the subcutaneous route.

[5] Additional tests with larger numbers of mice indicated that the MLD of sanocrysin by the intramuscular route is 1.5 mgm., and, therefore, only one and one-half as large as the intravenous MLD.

than by the intramuscular route without evidence of necrosis or other obvious injury at the site of inoculation.

While further work is necessary to determine the exact minimal therapeutic dose for a larger number of gold compounds administered by various routes, it is already evident from a study of the data obtained with intravenously injected sanocrysin and myochrysine that the factors which determine the therapeutic and toxic properties of a compound are not identical; the chemotherapeutic index for sanocrysin was approximately 2 as compared with 30 for myochrysine. Although there are as yet insufficient data for calculating the chemotherapeutic index for an "insoluble," organic gold compound, the preliminary observations on calcium aurothioglycolate point in the same direction, in that it exerted a striking curative effect, although after considerable delay, while even the largest amount that could be administered in a single dose gave rise to no signs of toxicity.

Several tests (table 2) with mice weighing less than 20 grams indicated, as was to be expected, that the size of the M.L.D. depends on the weight of the mouse but it should be noted that the relationship is not strictly proportional. For example, 10-grams mice tolerated 4 mgm. of myochrysine intravenously, while all 20-gram mice, which were given 8 mgm., died. In other words one cannot interpolate the toxicity of these compounds for animals heavier or lighter than 20 grams because, in proportion to weight, the heavier ones can tolerate less and the lighter ones more. It would, therefore, be misleading to describe toxicity in milligrams of compound per gram or kilogram of mouse. That being the case for mice of different age and weight it is clear that one cannot transpose the mouse-toxicity data to other animals or man simply by taking into consideration the difference in weight.

The data presented thus far have dealt with single-dose toxicity and studies on possible differences in excretion and cumulative effect of different compounds are still to be made. But it is already apparent that with the soluble gold compounds the cumulative effect is probably of greater significance as regards therapy than toxicity. Thus, an amount of myochrysine equiv-

TABLE 2

Toxicity of various types of gold compounds

COMPOUND	ROUTE	APPROXIMATE WEIGHT OF MICE	DOSE	NUMBER OF MICE	RESULT TIME OF DEATH IN DAYS
		grams	*mgm.*		
Gold chloride (acid) $AuCl_3 \cdot HCl \cdot 3H_2O$ (50% Gold)	Intravenous	20	5.0	4	D, D, D, D
			1.0	11	D, D, D, D, D, D, D, D, D, 1, 0
			0.75	6	D, D, D, D, D, D
			0.5	2	D, D
			0.5	6	D, 0.25, 0.25, 0, 0, 0
			0.5	6	D, 0, 0, 0, 0, 0
			0.5	6	1, 0, 0, 0, 0, 0
			0.2	2	D, 0
			0.2	4	D, D, S, 0
			0.2	6	0, 0, 0, 0, 0, 0
			0.2	6	0, 0, 0, 0, 0, 0
			0.1	25	All well
	Subcutaneous	20	15.0	6	Local necrosis; all survived
			10.0	6	Local necrosis; 1 dead 12th day
			5.0	6	Local necrosis; all survived
Gold chloride (adjusted to pH 7.0 with N/10 NaOH)	Intravenous	20	5.0	4	D, D, D, D
			4.0	6	D, D, D, 0.02, 0.02, 0.02
			3.0	16	(0.02) x 11, 2, S, S, 0, 0
			2.0	12	D, (0.02) x 9, 1, 1
			1.0	10	All well
Double thiosulphate of gold and sodium (Sanocrysin) $Na_3Au(S_2O_3)_2 \cdot 2H_2O$ (37% Gold)	Intravenous	20	20.0	2	0.004, 0.006
			10.0	3	0.17, 1, 1
			5.0	7	1, 1, 1, 1, 1, 1.5, S, 0
			4.0	4	1, 1, 1.5, 1.5
			3.0	4	1.5, 4, 5, S
			2.0	4	4, 5, S, S
			1.5	6	2, 4, 4, 6, 6, 18
			1.0	6	5, 5, 5, 7, S, 0
			0.8	6	All well
			0.5	3	All well
	Intramuscular	20	4.0	6	1, 2, 2, 2, 2, 5
			3.0	6	2, 2, 2, 2, 2, 3
			2.0	6	2, 5, 5, 5, 5, 7
			1.0	6	All well

D—died instantaneously; S—sick, but recovered; 0—appeared well. Additional toxicity tests with some of these compounds have yielded somewhat more accurate end-points, which are mentioned in footnotes to the text.

TABLE 2—*Concluded*

COMPOUND	ROUTE	APPROXIMATE WEIGHT OF MICE	DOSE	NUMBER OF MICE	RESULT TIME OF DEATH IN DAYS
		grams	*mgm.*		
	Intravenous	20	25.0	2	D, D
			10.0	8	D, D, D, D, D, D, D, D
			8.0	6	D, D, D, D, D, D
			7.0	6	D, 4, 0, 0, 0, 0
			6.0	6	5, 0, 0, 0, 0, 0
			5.0	3	All well
			4.0	6	All well
		10	10.0	3	D, D, D
			8.0	3	D, D, D
			6.0	3	D, D, D
Sodium aurothiomalate (Myochrysine)			4.0	3	0, 0, 0
$CH_2 \cdot COONa$	Intramuscular	20	50.0	4	0.25, 1, 1, 1
|			25.0	4	1, 1, 2, 5
Au—S—CH·COONa			10.0	10	3, 5, 5, S, S, S, S, S, 0, 0
(50% Gold)			8.0	6	5, 6, 7, S, S, S
			6.0	6	2, 0, 0, 0, 0, 0
			4.0	6	All well
		14	20.0	3	4, 6, 0
		14	10.0	3	6, 6, S
		11	6.0	7	2, 5, 6, 7, S, S, S
		13	5.0	7	5, 5, 6, 6, 7, 8, S
	Subcutaneous	20	*15.0*	6	5, 5, 7, S, S, S
			10.0	6	5, S, 0, 0, 0, 0
			8.0	10	S, S, 0, 0, 0, 0, 0, 0, 0, 0
	Intravenous	20	5.0	6	0.04, 0.04, 0.04, 2, 2, 0
			3.0	12	(0.02) x 3, (0.04) x 3, 4, S, S, 0, 0, 0
			2.0	6	1, 1, 4, S, 0, 0
Sodium aurothio-benzimid-azole-carboxylic acid (Triphal)			1.0	6	All well
(44% Gold)	Intramuscular	20	*10.0*	6	1, 1, 2, 7, S, S
			7.0	6	S, S, 0, 0, 0, 0
			5.0	4	All well
			3.0	4	All well
			1.0	4	All well
Calcium aurothioglycolate (Calaurol, Myoral)	Intramuscular	20	100.0	14	All well
$Au—S—CH_2 \cdot COO \rangle Ca$			50.0	3	All well
$Au—S—CH_2 \cdot COO \rangle$			40.0	10	All well
(67% Gold)			30.0	3	All well

alent to one or two M.L.D. was perfectly tolerated when injected in divided doses on alternate days over a period of one or two weeks. An amount of sanocrysin equivalent to 2 M.L.D. administered in 3 divided doses over a period of 4 days was harmless, even though the lethal effects of a single M.L.D. are delayed for 5 to 7 days. It would appear, therefore, that a part of the injected compound is excreted and another part stored and that while the therapeutic effect is determined by the amount stored in certain tissues, toxicity is more a function of the quantity of free chemically-reactive gold that is available at any one time.

MODE OF ACTION—EFFECT OF MYOCHRYSINE IN VITRO

The first question to be investigated in a study of the mode of action of these therapeutically active gold compounds is whether or not they can exert any effect *in vitro* on the microbial agent which is the cause of the experimental mouse arthritis. Myochrysine was selected for this study because it had the best chemotherapeutic index among the group. The possible direct effect of myochrysine on the microörganism was studied in a number of different ways, but no direct action was demonstrable by any of the methods.

(a) *Effect of various concentrations of myochrysine incorporated into solid medium.* Myochrysine in final concentrations of 1:1,000, 1:2,000, 1:5,000, 1:10,000, and 1:100,000, i.e. in concentrations of 100 mgm. to 1 mgm. per cent, was incorporated into 30 per cent ascitic fluid agar medium, and 0.05 ml. of culture was spread on the solidified medium in Petri dishes. With the exception of the 1:1,000 plates, in which there was marked precipitation of the medium, colonies developed as well on the agar containing myochrysine, even in concentrations by far exceeding those which might possibly obtain in the living animal, as on the control medium (table 3).

(b) *Effect of myochrysine in fluid media.* Since this microorganism cannot grow in the absence of a certain concentration of protein, the inhibitory or microbicidal effect of myochrysine in fluid medium had to be studied in broth containing at least 10

per cent of serum (i.e. about 0.6 to 0.8 per cent protein).[6] In one test a series of tubes containing 4.9 ml. of broth with 0.5 per cent glucose and 30 per cent ascitic fluid were seeded with 0.1 ml. of culture and myochrysine added in various amounts to reach final concentrations of 1:500, 1:1,000, 1:2,500, and 1:5,000. Although there was heavy precipitation in the 1:500 and 1:1,000 tubes and some precipitation in the other tubes, good growth occurred in all without evidence of inhibition or delay; proof of growth was obtained by Giemsa-stained films and subculture on fluid and solid media. Plating out of the various mixtures after 3 hours' incubation gave no indication that there might have been an

TABLE 3

Effect of various concentrations of myochrysine on growth of the type B microörganism of the mouse pleuropneumonia group in vitro

CONCENTRATION OF MYOCHRYSINE		GROWTH	
Mgm. per cent	Final dilution	Solid medium	Fluid medium
200	1:500		+
100	1:1000	0	+
50	1:2000	+	
40	1:2500		+
20	1:5,000	+	+
10	1:10,000	+	
5	1:20,000	+	
1	1:100,000	+	

initial microbicidal effect with subsequent growth resulting from a few surviving microorganisms. In another series of tests it was found that myochrysine in concentrations of 1 mgm. and 0.1 mgm. per 5 ml. of 10 per cent bovine serum broth (i.e. 1:5,000 and 1:50,000) does not prevent growth even when the inoculum contains minimal numbers of microorganisms. (See table 4.) Results similar to those shown in table 4 were also obtained when different amounts of a 48-hour culture were used as the inocula.

[6] After centrifugation and suspension in phosphate buffer of pH 7.35, these microorganisms survive for only a few hours at 37°C., and there was no evidence that addition of myochrysine in final concentrations of 1:1000 to 1:100,000 to such a suspension accelerated death of the microorganisms.

(c) *Effect of blood of myochrysine-treated animals.* Since there was no evidence that myochrysine, *per se*, in concentrations which may be considered equivalent to or larger than those which exert a distinct curative effect in the animal, either killed or prevented growth of the microörganisms *in vitro*, it was necessary to determine whether the blood of myochrysine-treated mice could exert such an effect. Preliminary tests with heparinized blood of mice, taken 5 hours after a single intravenous injection of 2 mgm., and with the blood of normal mice for comparison revealed no difference. In a more extensive study mice were given four intravenous injections of 2 mg. on alternate days and bled one day after

TABLE 4

Influence of size of inoculum on effect of myochrysine in vitro

DILUTION OF 24-HOUR TYPE B CULTURE USED AS INOCULUM	GROWTH IN 10 PER CENT BOVINE SERUM BROTH		
	No myochrysine	Myochrysine—2 mgm. per cent (1:50,000)	Myochrysine—20 mgm. per cent (1:5000)
Undiluted	+	+	+
10^{-1}	+	+	+
10^{-2}	+	+	+
10^{-3}	+	+	+
10^{-4}	+	+	+
10^{-5}	+	+	+
10^{-6}	+	+	0
10^{-7}	0	+	0
10^{-8}	0	0	0
10^{-9}	0	0	0

the last injection. The following test was performed: 0.45 ml. of blood of myochrysine-treated mice, or of blood of normal mice, or of heparinized bovine serum broth were mixed with 0.05 ml. amounts of varying dilutions of culture, ranging from undiluted through tenfold dilutions to 10^{-6}. After 2 hours' incubation at 37°C., 0.1 ml. of each mixture was spread on a bovine serum agar plate and 0.4 ml. was transferred to tubes of bovine serum broth. Since the disintegration of blood cells after a few days' incubation gives rise to a turbidity which prevents direct reading of cultures, the presence or absence of growth was determined by subculture on the days when the control tubes without

blood showed distinct growth. By these methods the blood of myochrysine treated mice showed no antimicrobial properties *in vitro.*

(d) *Effect of growing the microörganisms in a medium containing myochrysine on their capacity to produce arthritis.* The possibility that while myochrysine may not kill or inhibit the growth of these microörganisms on cell-free media, it might perhaps abolish their capacity to produce arthritis was investigated in the following manner: (a) The microörganisms were grown for 48 hours in 25 ml. of 10 per cent bovine serum glucose broth to which 5 mgm. of myochrysine were added; a similar culture without myochrysine was set up simultaneously. Both cultures were spun on the Swedish angle centrifuge for one hour at 4,000 r.p.m., the supernatant liquid discarded, and the sediment resuspended in 10 to 12 ml. of bovine serum broth. One-half milliliter of each culture was injected intravenously into each of 10 mice; 6 of the 10 mice inoculated with the myochrysine culture and 8 of the 10 with the normal culture developed arthritis. (b) The same experiment was repeated with the modification that the microörganisms were passaged in myochrysine-containing medium for 3 generations at 48-hour intervals. Ten mice inoculated with the resuspended microörganisms of the 3rd generation myochrysine culture, and 10 mice inoculated with "normal" resuspended culture, all developed equally severe, progressive arthritis. It is clear, therefore, that *in vitro* myochrysine (in a concentration of 20 mgm. per cent) not only does not inhibit growth in cell-free media but also does not modify the ability of these microörganisms to produce arthritis.

Thus, it was not possible to demonstrate any direct antimicrobial action *in vitro* for a compound which exerts a striking curative effect *in vivo.* Any supposed stimulation of the phagocytic mechanism which is sometimes attributed to gold compounds can hardly be considered as playing a part in the curative action of myochrysine on the experimental arthritis investigated here. For it should be recalled that normal mice, by phagocytosis or otherwise, can sterilize both the blood stream and the viscera after the intravenous injection of large amounts of this microör-

ganism, and existing evidence suggests that the arthritis is a result of their special affinity for, and intracellular invasion of, mesenchymal cells of the joints. Since a distinct curative effect can be obtained even when treatment is begun several weeks after the arthritis is well established, it would appear that the beneficial action of the chemical is exerted at a time when the etiological agent is already presumably established intracellularly. It is interesting to note, therefore, that a potent immune serum or vaccine administered at the very onset of arthritis and subsequently, did not possess the curative properties of the effective gold compounds, although injection of the immune serum just before infection could prevent the development of arthritis in practically all mice (Sabin and Morgan; 1940). Whether absorption of the chemical by the parasitized cells renders them unsuitable as hosts for the infecting microörganisms or the therapeutically effective compounds modify the pathological response to the infectious process, must still be investigated.

DISCUSSION

The experimental disease utilized in the present studies differs from other types of experimental arthritis, especially those caused by pyogenic microörganisms (whether bacterial or of the rat pleuropneumonia group) in that it is a progressive, chronic, essentially proliferative arthritis resembling human rheumatoid arthritis in its clinical and pathological manifestations more than any other experimental disease produced hitherto. The fact that this experimental arthritis in mice reacts to certain gold compounds in a manner that may be considered similar to the human disease, if the existing observations in man find further confirmation, provides on the one hand an experimental basis for human therapy, and on the other suggests enough basic similarity between the two conditions to warrant the use of the experimental disease for further exploration in the field of therapy. The following data obtained in the present study would appear to have a bearing on the use of gold compounds in human therapy:

(1) The gold compounds exerting a curative effect may be

inorganic, or organic and soluble or insoluble, but in the colloidal state gold had no therapeutic value.

(2) The greatest and most rapid response was obtained with the largest doses of the soluble compounds. The same total dose administered over a period of a week gave better results than when it was given in individual smaller doses over a longer period of time. For example, 2.5 mgm. of the double thiosulfate of gold and sodium administered on alternate days in 5 doses of 0.5 mgm. each, brought about an excellent therapeutic response, while the same amount of compound given in 0.25 mgm. doses over a period of 3 weeks had little or no therapeutic effect. The present practice in human therapy of administering gold compounds at weekly intervals may actually prevent or diminish optimum effect.

(3) With borderline doses of therapeutically effective compounds there is a high incidence of recurrence after the cessation of treatment.

(4) The earlier in the disease treatment was begun, the more rapid and complete was the therapeutic response. When treatment was delayed for as long as 4 weeks (this coincides approximately with the stage when distinct cartilage destruction is first observed in the mouse arthritis) the involvement of some joints, although not all, had progressed to a point where therapy was no longer effective. The necessity of evaluating this therapy in early cases of human rheumatoid arthritis is thus emphasized.

(5) Tests with calcium aurothioglycolate, an insoluble organic gold compound, indicated that while it is tolerated in very large doses, enough active substance is absorbed over a period of weeks to bring about a complete and persistent curative effect. The desirability of investigating the obvious applications of such a compound in the human disease, which progresses very much more slowly than the mouse arthritis, is self-evident.

(6) Toxicity and therapeutic effectiveness of the gold compounds, studied thus far, are not parallel. Thus, the double thiosulfate of gold and sodium (sanocrysin) had a chemotherapeutic index of approximately 2 for the intravenous route, while

under identical conditions the index for sodium aurothiomalate (myochrysine) was 30.

All chemotherapeutic agents, including the arsphenamine and sulfonamide compounds, have a certain degree of toxicity and danger associated with their use, and, therefore, the probable benefits to be derived must always be weighed against the possible dangers involved. Whether the incidence of serious toxic reactions with suitable gold compounds is greater than with certain other chemotherapeutic agents now used in human therapy is problematical. However, while among the compounds we have tested thus far there is already one, sodium aurothiomalate (myochrysine) with a chemotherapeutic index of approximately 30, it is not unlikely that other gold compounds exist or may be synthesized with a still greater margin of safety. This assumption is based on the demonstration in the present study that different factors are involved in determining the minimal lethal dose and the minimal therapeutic dose of a compound. Further work, therefore, will concern itself not only with a systematic study of the relationship between chemical structure and the chemotherapeutic index of existing and newly synthesized gold compounds, but also with a search for other chemical agents with similar curative properties.

SUMMARY

1. Certain inorganic and organic gold compounds, of both the aliphatic and aromatic series, have been found to exert a curative effect on an experimental, proliferative, chronic arthritis in mice produced by a filtrable microörganism of the pleuropneumonia group which is a normal inhabitant of the upper respiratory tract of mice.

2. While colloidal gold compounds were ineffective, tests with an "insoluble," organic compound (calcium aurothioglycolate) revealed that enough active substance is absorbed over a period of weeks to bring about a complete and persistent curative effect.

3. The greater the dosage of an effective compound the more rapid and complete is the disappearance of the arthritis. A given total dosage which is curative when administered in a small

number of divided doses within a short period, may be ineffective when it is divided in a large number of small doses and given over a much longer period of time.

4. With doses on the borderline of effectiveness there is a high incidence of spontaneous recurrence after cessation of treatment.

5. The earlier in the disease treatment is begun, the more rapid and complete is the therapeutic response. When treatment was delayed for 4 weeks after the onset of arthritis, the involvement of some joints in the majority of mice had progressed to a point where therapy was no longer effective.

6. Arthritis disappeared completely in 96 per cent of 171 mice which were treated with suitable gold compounds in adequate dosage and at the proper time, but only in 5 per cent of 77 mice which were treated with other compounds such as colloidal gold, bismuth subsalicylate, sodium salicylate, neoarsphenamine or sodium sulfapyridine, and in only 7 per cent of 70 mice which remained untreated.

7. Neither the therapeutically effective compound *per se*, nor the blood of mice treated with it in large doses prevented the growth of the etiological agent *in vitro*; furthermore, microörganisms grown in the presence of an active gold compound for 3 generations did not lose their capacity to produce arthritis.

8. Toxicity and therapeutic effectiveness were found to depend upon different properties of the same compound. Thus, the margin of safety as represented by the chemotherapeutic index $\left(\frac{\text{minimal lethal dose}}{\text{minimal curative dose}}\right)$ varied from approximately 2 for the thiosulfate of gold sodium (sanocrysin) to about 30 for sodium aurothiomalate (myochrysine).

REFERENCES

COLLIER, W. A. 1939 Zur Goldtherapie der experimentellen Polyarthritis der Ratte. Z. Immunitäts., **95**, 132–138.

FINDLAY, G. M., MACKENZIE, R. D. AND MACCALLUM, F. O. 1940 Chemotherapeutic experiments on pleuropneumonia-like organisms in rodents. Brit. J. Exptl. Path., **21**, 13–22.

FORESTIER, J. 1929 L'aurothérapie dans les rhumatismes chroniques. Bull. mém. soc. méd. hôp. Paris, **53**, 323–327.

HENCH, P. S., BAUER, W., DAWSON, M. H., HALL, F., HOLBROOK, W. P. AND KEY, A. 1939 The problem of rheumatism and arthritis (Fifth Rheumatism Review). Ann. Intern. Med., **12**, 1005–1104.

SABIN, A. B. 1939a Experimental proliferative arthritis in mice produced by filtrable, pleuropneumonia-like microorganisms. Science, **89**, 228–229.

SABIN, A. B. 1939b Mice as carriers of pathogenic pleuropneumonia-like microorganisms. Science, **90**, 18–19.

SABIN, A. B. 1940 Joint pathology at different stages of experimental, proliferative, progressive arthritis in mice. J. Bact., **39**, 343. (Abstract).

SABIN, A. B. AND MORGAN, I. M. 1940 Effect of immune serum and vaccines on experimental arthritis produced by the filtrable microorganisms of the mouse pleuropneumonia group. Arch. Path. **29**, 735. Abstract.

SWIFT, H. 1937 A simple method for preserving bacterial cultures by freezing and drying. J. Bact., **33**, 411–421.