tents. The difference was in both relative and absolute amounts. Biologic false positive sera from presumably normal individuals showed differences from normal sera which were qualitatively similar to those of syphilitic sera but of considerably smaller magnitude. However, biologic false positive sera from individuals known to have diseases other than syphilis gave findings essentially like those obtained with syphilitic sera. No unique components and no significant mobility differences from normal sera were seen among the serum protein constituents of either the syphilitic or biologic false positive sera.

(2) *Fractionation.* The distribution of the reactive antibodies among the various serum components has been determined by (a) fractional precipitation with increasing concentrations of ammonium sulfate, under standardized conditions; and (b) isoelectric precipitation of the globulins by treatment of the diluted sera with CO_2. The latter method offers the advantage that it does not require dialysis and that it yields a single, strongly reactive fraction containing preponderantly *gamma* globulin (protein concentration 4–8 mg per cc). Analyses made on some 200 syphilitic and biologic false positive sera revealed that (a) fractions GI and GII, precipitable, respectively, by 1.4 and 1.7 M ammonium sulfate, contained most of the serological activity, whereas the crude albumin, remaining after precipitation of GIII by 2.1 M ammonium sulfate, was always serologically inactive; (b) the sum total of the individual titers of the isolated fractions of syphilitic sera, prepared by either of the above methods, was consistently less than that of the whole serum, whereas with biologic false positive sera, elimination of the crude albumin resulted in a significant increase in the titer of the globulins over that of the parent sera. This differential behavior, particularly pronounced with weakly reactive sera, suggested an inhibitory effect of crude albumin on the reaction of antibodies of biologic false positive sera with lipoidal antigen.

(3) *Inhibition and redispersion.* It was found that addition of the crude albumin fraction to globulin fractions derived from false positive sera causes complete inhibition of specific flocculation with lipoidal antigen, as well as redispersion of floccules formed before crude albumin was added. The addition of this fraction had little effect on the reaction of globulins isolated from syphilitic sera. So far the heat-stable inhibiting component of the albumin fraction has been found only in human sera, though individual preparations vary in potency. Crystalline human serum albumin does not exert any inhibitory effect. Further work on the isolation and identification of the inhibitor is in progress.

(4) *Heat stability.* Experiments were made with some 50 syphilitic and biologic false positive sera, or their isolated globulin fractions, by heating the samples for 20 minutes at temperatures ranging from 56 to 66° C. The data indicate that the antibodies of false positive sera are more susceptible to heat inactivation than those of syphilitic sera. The presence of non-specific serum proteins appears to be without effect on the temperature-activity relation.

(5) *Adsorption on calcium phosphate.* A considerable degree of purification of the syphilitic antibody has been achieved by adsorption from whole sera on freshly precipitated calcium phosphate, followed by precipitation of the eluate with ammonium sulfate.[7] This procedure has yielded under optimal conditions a solution containing 0.015 mg of protein nitrogen per cc per 4 flocculation units. While about 80 per cent. of the total antibody activity of syphilitic sera of varying titer is adsorbed by the calcium phosphate, in a limited number of experiments with false positive sera, no activity has been found in the eluate.

The observations described herein indicate that the antibodies of truly syphilitic sera, reactive with lipoidal antigen, differ from those of biologic false positive sera in certain chemical and immunological respects. The possibility of the application of these findings to the development of a practical method of differentiation is being explored.

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A NEW TRYPANOCIDAL AGENT: γ-(p-ARSENOSOPHENYL)-BUTYRIC ACID[1]

γ-(p-ARSENOSOPHENYL)-BUTYRIC acid was first described in a paper from this laboratory in 1940[2] It is a stable white crystalline powder, slightly soluble in water, but readily soluble in dilute alkali. Neutral solutions of the sodium salt can be sterilized by autoclaving, and such sterile solutions in sealed ampules

[7] H. Neurath, F. W. Putnam, E. Volkin and J. O. Erickson, Abstracts of papers presented at the one hundred and eighth meeting, American Chemical Society, New York, N. Y., September, 1944, p. 21B.

[1] Preliminary report.

[2] G. O. Doak, H. G. Steinman and H. Eagle, *Jour. Am. Chem. Soc.*, 62: 3012, 1940.

have been kept at room temperature for 12 months without demonstrable change.

The toxicity and trypanocidal activity of this compound against *T. equiperdum* have been described in a previous communication.[3] In both mice and rabbits, the chemotherapeutic index was significantly higher than that of tryparsamide; and the relatively small curative dose ($CD_{50} = 1.6$ mg per kg in mice and 3.6 mg per kg in rabbits) suggested that treatment in man might be effected with a considerable economy in time and cost.

Of the species of trypanosomes pathogenic for man, cultures of *Schizotrypanum cruzi* (the cause of Chagas disease), which is relatively resistant to tryparsamide and most other arsenicals, were found to be immobilized and killed *in vitro* within 4 hours by dilutions in excess of 1:400,000.[4] In other laboratories, the compound was found to be effective against *T. rhodesiense*[5] in mice, and Van Hoof, Henrard and Peel[6] obtained encouraging results in the treatment of *T. gambiense* infections in guinea pigs, chimpanzees and man.

Of particular therapeutic significance was the finding that the γ-(p-arsenosophenyl)-butyric acid was fully active against so-called "arsenic-resistant" strains of trypanosomes. (a) A variant of *T. equiperdum,* which was 5 to 200 times more resistant to amino- and amide-substituted arsenicals than the parent strain, was found to be normally susceptible to the butyric acid compound *in vitro.*[7] (b) *Schizotrypanum cruzi* infections in man are known to resist treatment with tryparsamide and other arsenicals; but as noted above,[4] a typically "arsenic-resistant" culture of that organism proved susceptible to the γ-(p-arsenosophenyl)-butyric acid *in vitro.* (c) The curative dose in *T. rhodesiense* infections in mice[5] was identical with the curative dose in experimental *T. gambiense* or *T. equiperdum* infections, despite the fact that the first-named species in man is reported to be more resistant to treatment than *T. gambiense.* (d) In both experimental animals and man, Van Hoof, Henrard and Peel[6] observed that strains of *T. gambiense* resistant to tryparsamide were susceptible to the butyric acid-substituted phenyl arsenoxide.

In the light of these experimental and clinical data, a large-scale field trial was begun in the summer of 1944, with the collaboration of the Sleeping Sickness Services of the Gold Coast (Dr. G. Saunders), Nigeria (Dr. J. L. McLetchie), Belgian Congo (Major General L. Van Hoof) and the British Forces in West Africa (Brigadier G. M. Findlay). Similar studies are projected in Northern Rhodesia (Dr. J. F. C. Haslam), French West Africa (Colonel G. LeRouzic), French Equatorial Africa (Colonel Ceccaldi) and Sierra Leone (Dr. R. D. Harding). The wide geographic distribution of the cases assured the inclusion of a variety of strains, perhaps differing in virulence and in their resistance to treatment.

The schedule of treatment and the total dosage of drug were deliberately varied in the several treatment centers in order to determine the minimum curative dose and the most efficient and practicable method of administration. The drug was supplied as an ampuled sterile 2 per cent. solution. This was injected either twice weekly, three times weekly or daily, to a total of 6 to 24 injections. The individual dose was similarly varied between 0.25 and 0.5 mg per kg, the average individual dose being 0.8 to 1.5 cc of the 2 per cent. solution. The latter dose in a man weighing 60 kg represents 1/50 of the maximal tolerated dose in mice and 1/8 of the maximal tolerated dose in rabbits.[3] The recommended method of administration was intravenous; but the compound has been injected intramuscularly in infants and in patients with small veins, with no untoward reaction except for transitory pain at the site of injection.

To date, more than 200 patients have been treated in collaboration with the workers previously listed, and it is anticipated that the study will ultimately include in excess of 1,000 patients. Of necessity, this must be a preliminary report, since the evaluation of therapeutic efficacy, even in early cases, presupposes observation for at least one year after the conclusion of treatment. Nevertheless, the following tentative conclusions seem justified by the results obtained to date.

(a) Except for occasional nausea and vomiting, observed after approximately 2 per cent. of the injections, there have been no toxic reactions in more than 100 early cases, representing a total of more than 800 injections.

(b) When organisms were present in the lymph nodes or blood, these regularly disappeared within 30 to 60 minutes after the first injection (*cf.* Table 1). This included some patients previously shown to be infected with a strain of trypanosomes resistant to tryparsamide.

(c) There is reason to believe that in *early* cases, without central nervous system involvement, sterilization and definitive cure can be obtained within less than 2 weeks, by daily injections of approximately 0.4 mg per kg each. The first four cases of early

[3] H. Eagle, R. B. Hogan, G. O. Doak and H. G. Steinman, *Public Health Reports,* 59: 765, 1944.
[4] H. Eagle and H. J. Magnuson, unpublished data.
[5] C. M. Scott, personal communication.
[6] L. Van Hoof, C. Henrard and E. Peel, personal communication.
[7] H. Eagle and H. J. Magnuson, *Jour. Pharm. and Exper. Therap.,* in press.

TABLE 1

THE RATE AT WHICH TRYPANOSOMES DISAPPEARED FROM LYMPH NODES AFTER TREATMENT WITH γ-(P-ARSENOSOPHENYL)-BUTYRIC ACID

Time of second node puncture, minutes after treatment	Number patients tested[1]	Result of second lymph node puncture	
		Positive	Negative
30	21	3[2]	18
45	15	1	14
60	2	0	2
Untreated controls, repunctured 60 minutes after the original positive puncture	16	14	2

[1] None of these are duplicates: all nodes were positive just before treatment.
[2] In two of these patients repunctured 60 minutes after treatment, no organisms could be found.

infection treated with this compound by Dr. L. Van Hoof, each of whom received a total of only 3.5 to 4.5 mg per kg, distributed over 6 to 11 injections, have now been observed for periods of 1, 5, 6 and 8 months. They are clinically well, the blood and lymph nodes remain free of organisms, and the spinal fluid remains normal. It is to be noted that one of those four patients was infected with a strain of *T. gambiense* which was unaffected even by large doses (6 gm) of tryparsamide.

(d) In the *late* cases, with clinical and laboratory evidence of central nervous system involvement, the results to date are not encouraging. A few of the patients have improved; the majority have been unaffected. There is some indication also that the drug is more toxic in late cases, in that two of these patients have died with cerebral symptoms suggestive of a toxic encephalopathy. The treatment of advanced cases is continuing, at reduced dosage (0.25 mg per kg); and it is planned to treat some cases with small doses of the γ-(p-arsenosophenyl)-butyric acid in conjunction with a pentavalent arsenical. The therapeutic efficacy of the arsenoxide alone in cases with an altered spinal fluid, but without clinical evidence of central nervous system involvement, remains to be determined.

(e) In collaboration with Brigadier G. M. Findlay, of the British Forces in West Africa, and Dr. J. Simpson, veterinarian of the Accra District, Gold Coast, a few sheep and cattle with clinical evidence of trypanosomiasis, confirmed by blood examination, and one horse with advanced symptoms have been treated. The sheep and the horse improved markedly after 2 to 3 injections at approximately 0.25 mg per kg each, and at the present writing seem well after a total of 6 to 8 injections. Organisms persist in the cattle despite some clinical improvement.

In summary, the results to date suggest that γ-(p-arsenosophenyl)-butyric acid is a highly active trypanocidal agent, with which it may be possible to cure early cases of human trypanosomiasis within two weeks or less, and with reasonable freedom from toxic reactions. This, coupled with the fact that it appears to be active against strains of trypanosomes resistant to tryparsamide and other arsenicals, may greatly simplify the mass treatment of the disease, and permit a considerable economy of time and cost. The results in late cases are not encouraging. There is some indication that the compound may be effective against some, but not all, forms of animal trypanosomiasis.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A MICROCALOMEL ELECTRODE FOR POLAROGRAPHIC MEASUREMENTS

A SATURATED calomel electrode is generally used as an external electrode in polarographic measurements. Kolthoff and Lingane[1] describe a special H-cell for use in making half-wave potential measurements. They also describe various salt bridges for use in connecting electrolysis cells to permanent external anodes. Hume and Harris[2] have discussed a special salt bridge for use in connecting an external calomel electrode with the solution being studied in the electrolysis cell.

We have designed a microcalomel electrode (Figs. 1 and 2) to be used internally with the dropping mercury electrode. An obvious advantage of using the micro electrode is that it is independent of the electrolysis vessel which can therefore be cleaned and refilled with fresh samples without disturbing the electrode itself. When desired, the calomel electrode and its salt bridge container can be removed and stored in saturated potassium chloride solution.

The electrical resistance of a cell composed of a microcalomel electrode and a hydrogen electrode dipping into a saturated solution of potassium chloride was found by standard methods[3] to be 1,500 ohms. The same resistance was found for the cell irrespec-

[1] I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, Inc., New York, N. Y., 1941.
[2] D. N. Hume and W. E. Harris, *Ind. Eng. Chem., Anal. Ed.*, 15: 465, 1943.
[3] S. G. Starling, "Electricity and Magnetism," sixth edition, Longmans, Green and Company, New York, N. Y., 1937.