swine serum, exhibited varying degrees of anaphylactic shock. This was characterized by dyspnea, coughing, sneezing, roughening of the coat, and prostration. A moderate to marked hyperemia and edema of the conjunctivæ also developed. Swine, rabbit, goat, and horse serum each caused approximately the same degree and type of allergic reaction in specifically sensitized animals regardless of whether the initial, sensitizing dose had been inhaled or given parenterally. Of 56 sensitized animals which were treated in this manner, 3 died in the chamber during exposure to an atomized specific antigen. Two of these had been sensitized by a single intraperitoneal injection of 0.3% crystalline egg albumin, the third by inhalation of the same substance. Other than this, however, anaphylactic reactions were sub-lethal and symptoms usually subsided within a few hours after each exposure to aerosol. Control animals gave slight or no reactions. Four weeks after sensitization by inhalation of various antigens, 14 guinea pigs were injected intravenously with 0.3 to 0.5 cc of the specific antigen. Twelve of the 14 animals in this group died of typical anaphylactic shock.

It has been suggested that repeated inhalations of small doses of anti-influenzal serum might be used prophylactically. A second series of experiments, using goat serum alone, was conducted to determine whether or not widely spaced inhalations of smaller quantities of serum would also produce a state of active hypersensitivity. Guinea pigs and rabbits were exposed to aerosols of undiluted or diluted (1:10) goat serum for 20 minutes at weekly intervals. Upon the third of the weekly exposures, mild reactions were observed. By the fifth week of such treatment, allergic reactions were quite marked and were in every way similar to those previously described.

Histologic studies of tissues from animals which were autopsied two days following the last of several (5 or 6) 20-minute exposures to atomized antigen revealed acute inflammatory changes in lungs, trachea, and nasal mucosæ. These were present to some extent in all of the animals, including controls, but were more pronounced in those which had been previously sensitized by injection or inhalation of the specific antigen.

From these observations it is apparent that active sensitization can be readily accomplished by inhalation of finely atomized fluid antigen and that serious allergic reactions, even fatal anaphylactic shock, may occur when hypersensitive animals inhale aerosol of specific antigen.

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Experimental Basis for the Chemotherapy of *Trichomonas vaginalis* Infestations. I.

GARTH JOHNSON AND RAY E. TRUSSELL. (Introduced by E. D. Plass.)
From the Departments of Obstetrics and Gynecology, and of Hygiene and Preventive Medicine, State University of Iowa, Iowa City, Ia.

Clinical reports clearly indicate that an entirely satisfactory therapeutic agent is not established for the treatment of *Trichomonas vaginalis* infestations. *In vitro* studies of the trichomonacidal effect of various compounds can supply only collateral data for future therapeutic trial. Such factors as prolonged exposure of the parasite to the medication mixture, the intervention of tissue exudates, and preparatory cleansing and drying of the site of infection lead to divergence of *in vitro* and clinical findings.

The data reported in this paper were obtained from tests of a number of proprietary compounds which were solicited from the manufacturers, as well as others selected for reasons of theory or convenience. The list will be enlarged in future studies by investigation of many compounds suggested by clinical literature and will be supplemented by

reports dealing with the inhibiting action of these test preparations.

The completion of this study was made possible by the generous interest and support of Doctor E. D. Plass and Doctor M. E. Barnes.

Method. Various dilutions of the test compounds were made up in sterile, cotton stoppered tubes to a final volume of 3.8 cc. A human serum content of 25% was established either by adding 1 cc of 100% serum to 2.8 cc of drug solution or by dissolving the drug directly to the final dilution in 25% serum. In the first method the drug solution was made up to a calculated concentration such that the addition of 1 cc of serum and 0.2 cc of inoculum would reduce it to the required final dilution. A useful formula for computing the dilutions is given in Appendix B. In either case the serum was previously adjusted to pH 6 with N/1 HCl. These solutions were prepared aseptically and throughout the test period they were maintained at 37°C in a constant temperature bath. The final volume of the test mixture was 4 cc and consisted of 0.2 cc of inoculum, plus 2.8 cc of drug solution and 1 cc of 100% serum, or 3.8 cc of 25% serum containing the drug in the required final dilution.

An inoculum of 400,000 organisms was introduced by adding 0.2 cc of a 48-hour

TABLE I.
Compounds Without Demonstrated Trichomonacidal Activity.

Chemical name	Synonym or trade name	Max. conc., one part in:	Max. exposure, min.	pH Test mixture	pH Control	Solvent
7-iodo-8-hydroxyquinoline-5 sulfonic acid	Chiniofon Yatren	70	10	6.2	6.36	Water
5,7,-diiodo-8-hydroxyquinoline	Diodoquin	(70% sat.)*	120	5.5	5.62	”
		6% suspension	10			
Bile Salts		70	60	6.18	6.3	”
p-Carbamyl-aminophenyl-arsonic acid	Carbarsone Arsanilic acid	100	120	7.79	5.27	N/1 NaOH
Acetarsone + boric acid + CHO	Devegan	(70% sat.)*	60	5.3	5.47	Water
2,4-dihydroxy-n-hexylbenzene	Caprokol Hexylresorcinol	2800 (see Table II)	10	5.94	5.92	”
Triiodomethane	Iodoform	(70% sat.)*	15	6.5		”
Triacetylpyrogallol	Lenigallol	10% suspension	10	5.1	6.36	”
	Meta Cine	(70% sat.)*	15	3.11	5.78	”
		6% suspension	10			
Sodium perborate + hydroxyquinoline + aluminum silicate + sodium borate + boric acid	Pulvis alkalinus fungi	(70% sat.)	60	8.82	5.5	”
Quinine sulfate		1000	60	5.43	5.47	”
Sodium anthraquinone-sulfonate		140	60	6.1	6.3	”
Sodium 2,5-bisulfanil-amidobenzene-sulfonate		1000	15	5.6	5.38	”
Sodium N′-cinnamoyl-sulfanilamide		1000	15	5.8	5.38	”
Sulfadiazine		14000	15			”
Sulfaguanidine		750	60	5.5-6.0	5.47	”
Sulfamethyldiazine		4700	60	5.5	5.47	”
Sulfanilamide		178	180	5.8	5.58	”
Sulfapyridine		3500	60	5.4	5.47	”
Sulfathiazole		1100	60	5.57	5.6	”
Sulfathiazole + *B. lactose*		(70% sat.)*	60	5.69	5.71	”
Alkyl dimethyl-benzylammonium chlorides	Zephiran	1400	15	6.2		”

* Sat.—Saturated.

TABLE II.
Trichomonacidal Compounds.

Chemical name	Synonym or trade name	Dilution range* killing in 10 min. but not in 5.† 1 part in:	pH	
			Test mixtures	Control
Acetylamino-hydroxyphenyl-arsonic acid	Stovarsol, Acetarsone, Spirocid	20	8.99	6.0
	Aerosol	500	5.27	6.36
Sodium methylene-sulfonamino-hydroxy-phenyl arsonate	Aldarsone	140-150	6.2-6.3	5.9-6.1
3,3′-diamino-4,4′-dihydroxy-arseno-benzene dihydro-chloride	Arsphenamine, Salvarsan, etc.	3100-3400	5.3-5.8	6.05
Boric acid		28 (in 90 min.)	5.62	6.4
Colloidal chlorthymol	Clymocol	2200	6.28	6.36
"Meta-dihydroxy-di-secondary hexyl benzene"	Dihexylin Aqueous Tincture	5800	3.64	6.30
		5500-6000	5.0	6.57
Diiodoquinone + boric acid + lactose + dextrose	Floraquin	70% saturated (in 30 min.)	5.4-5.8	5.9
Diiodoquinone + boric acid + lactose without dextrose		70% saturated (in 30 min.)	5.6	5.9
Emetine hydrochloride		36	5.7	6.36
Isoamyl-hydrocupreine hydrochloride	Eucupin Base (in N/1 HCl adjusted)	250	5.3	6.36
1-n-hexyl-2,4-dihydroxy-benzene	Hexylresorcinol	Slight excess in emulsion	6.01	6.09
Mercuric oxycyanide		7200-7700	6.1-6.2	6.05
"Polymerized disulfonic-dioxy-dimethyl-diphenyl-methane acids"	Negatan	120-140 stock sol, or 270 active ingredients	1.76-2.16	
N′-benzoylsulfanil-amide		10% saturated (in N/1 NaOH)	10.2-10.8§	5.9
N′-furfurylsulfanil-amide		8% saturated (in N/1 NaOH)	10.7§	5.9
Sodium 3,3′-diamino-4,4′ dihydroxy-arseno-benzene-N-methanal sulfoxylate	Neoarsphenamine	2000	6.12	6.09
Ethyl hydrocupreine hydrochloride	Optochin Base (in N/1 HCl, adjusted)	142 (in 45 min.)	4.82	6.3
"P-t-octyl-phenyl-di-ethoxy-dimethyl-benzyl-ammonium-chloride"	Phemerol	1600	5.98	6.0
Phenol	Carbolic Acid	210	5.9-6.2	6.4
Phenylmercuric acetate		40,000	5.81	5.82
Phenylmercuric benzoate		36,000-38,000	5.8-6.0	5.84
Phenylmercuric chloride		34,000-38,000	5.8-6.0	5.99
Phenylmercuric nitrate		36,000-38,000	5.77-5.8	5.8
Quinine hydrochloride		100	6.3	6.57
Saponin		200	6.27	6.57
Silver picrate	Picrotol	14,000-17,000	6.08-6.13	6.1
Sulfadiazine Pickrell Solution	3% sulfadiazine in 8% triethanolamine	Triethanol-amine 5875		
		Sulfa-diazine 1566	8.66	5.60

TABLE II—Continued.
Trichomonacidal Compounds.

Chemical name	Synonym or trade name	Dilution range* killing in 10 min. but not in 5.† 1 part in:	pH	
			Test mixtures	Control
Sulfanilylacetamidine		400 (in N/1 NaOH)§	10.05	5.9
Sulfanilamido-thymol		250 (in N/1 NaOH)§	10.29	5.9
Disodium 3,3'-diamino-4,4'-dihydroxy-arseno-benzene-N-dimethylene sulfonate	Sulpharsphenamine‡	3000-4000	6.0-6.2	6.2
Potassium antimonyl tartrate	Tartar emetic‡	1600-2000	5.63-6.0	6.2
Sodium bismuth thioglycollate	Thiobismol‡	200-300	6.9-7.32	6.3
Trihydroxytriethyl-amine	Triethanolamine	20	9.78	
Sodium N-phenyl-glycine-amide-p-arsonate	Tryparsamide	175	6.3	6.57
5-chloro-7-iodo-8-hydroxy-quinoline	Vioform	70% saturated (30 min.)	6.3-6.5	6.57
		10% emulsion	5.88	6.57
Isoctylhydrocuprein dihydrochloride	Vuzin dihydro-chloride	2000	5.81	6.57
Zinc acetate (Analytical)		140 (45 min.)	5.22	6.3

* Slight differences in cultures necessitate expression of a killing range instead of a killing point.
† Dissolved in water unless otherwise designated in column 3.
‡ Compounds of arsenic, antimony, and bismuth gave variable results.
§ Activity may be due to the elevated pH.

bacteria-free culture[1,2] containing 2 million organisms per cubic centimeter. The population was determined by hemocytometer count and was reduced, if necessary, by dilution with sterile, modified Ringer's solution or culture fluid. Thorough mixing was then effected by gentle shaking and air agitation by means of a pipette controlled by mouth. The same procedure assured that all test organisms were washed from the upper level of the test tube wall.

At intervals of 5 and 10 minutes 0.2 cc of the test mixture containing trichomonads was transferred by a sterile pipette to 10 cc of C.P.L.M.* medium adjusted to pH 6.0.[3] (See Appendix A). Glass electrode determinations of the pH of the medication mixtures and an inoculated control containing 2.8 cc of distilled water and 1 cc of serum were then recorded.

The subcultures were examined for evidence of multiplication after 6 and 9 days. The effective concentration of the test compound is arbitrarily accepted as that dilution, or dilution range, which kills the protozoa in 10 but not in 5 minutes. Failure of the trichomonads to multiply after transfer to the C.P.L.M. medium indicated that they were killed by the test mixture.

A high protein content and an acid reaction were employed in the test mixture in order to simulate the conditions to be encountered in clinical application of the drug to the infected vagina. The validity of the test procedure is based in part upon data showing that C.P.L.M. medium* will support a high population of trichomonads after 9 days incubation when inoculated with 1 or 2 organisms. When 0.2 cc of medication mixture was transferred to the same medium the inoculum contained 2,000 treated organisms. Growth of the protozoa in the medium, as determined by inspection after 9 days incubation, indicated that more than 0.01% of the organisms had survived exposure to the medicament.

In summary the test procedure involved the addition of 400,000 trichomonads to a battery

1 Trussell, R. E., *J. Iowa M. Soc.*, 1940, **31**, 66.
2 Trussell, R. E., and Plass, E. D., *Am. J. Obst. and Gynec.*, 1940, **45**, 220.
* Cysteine-peptone-liver infusion-maltose.
3 Johnson, G., *J. Parasitol.*, 1942, **28**, 369.

of drug dilutions having in each case a volume of 4 cc and containing 25% human serum adjusted to pH 6. At intervals of 5 and 10 minutes 2,000 treated organisms contained in 0.2 cc of the test mixture were transferred to 10 cc of sterile C.P.L.M.* medium. Observation of these cultures by microscope after 6 and 9 days revealed whether or not the treated organisms had multiplied. Failure to multiply indicated that the trichomonads had been killed by exposure for a measured time interval to a given dilution of a drug.

Results. The data obtained from duplicate or triplicate tests of the same dilution range are summarized in Tables I and II.

Summary. 1. A standardized test procedure employing a highly favorable culture medium has been employed to test the trichomonacidal action of a number of recommended and suggested drugs. 2. Inspection of the data in Tables I and II suggests an approach which may lead to a more satisfactory therapeutic agent than is now available for the treatment of *Trichomonas vaginalis* vaginitis. 3. The following compounds in dilutions of 1:1000 or more killed trichomonads *in vitro* in less than 10 minutes: arsphenamine, clymocol, dihexylin, mercuric oxycyanide, neoarsphenamine, phemerol, phenylmercuric acetate, phenylmercuric benzoate, phenylmercuric chloride, phenylmercuric nitrate, silver picrate, sulfadiazine, sulfarsphenamine, tartar emetic, vuzin dihydrochloride.

APPENDIX A.

The C.P.L.M medium was prepared as follows:

32 g Bacto peptone
1.6 g Bacto agar
2.4 g cysteine monohydrochloride
1.6 g maltose[4]
320 cc Bacto liver infusion (prepared by manufacturer's instructions)
960 cc modified Ringer's solution (NaCl, 0.6%; $NaHCO_3$, $CaCl_2$, KCl, 0.01%)
Add N/1 NaOH to pH 6 (approximately 11 cc)
Keep in boiling water bath until agar is dissolved, then filter through coarse paper, add 0.7 cc 0.5% methylene blue
Tube in 8 cc volumes, autoclave, allow to cool.
Add 2 cc sterile, undiluted human serum.

The final mixture has the following percentage composition:

By volume—serum 20%, Ringer's solution 60%, liver infusion 20%.
By weight—cysteine monohydrochloride 0.15%, agar 0.10%, peptone 2.0%, maltose 0.10%, methylene blue 0.0002%.

This medium is made anaerobic[3] by the cysteine with the methylene blue serving as an indicator.

APPENDIX B.

The volume of water (H) required to dilute 2.8 cc of stock solution is determined as follows:

$$H = \frac{1.96D}{d} - 2.8,$$

where D is the final dilution desired after adding 1 cc of serum and 0.2 cc of inoculum; d is the dilution in the stock solution. After mixing, this same volume of fluid was withdrawn and discarded before adding the serum.

[4] Trussell, R. E., and Johnson, G., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 176.

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Biochemical Findings in Normal and Trauma-Resistant Rats Following Trauma.

A. H. NEUFELD, C. GWENDOLINE TOBY, AND R. L. NOBLE. (Introduced by J. B. Collip.)

From the Research Institute of Endocrinology and the Department of Biochemistry, McGill University, Montreal.

A method for traumatizing rats in a rotating drum has previously been described, whereby trauma can be administered quantitatively, the percentage mortality being directly related to the degree of trauma.[1] Animals which succumb following this treatment show a picture typical of shock. By gradually increasing the amount of trauma, it was found that animals would tolerate 1000 turns, an amount fatal to the normal animal.[2] In view of the striking differences between the normal and the resistant rat subjected to trauma, chemical analyses were done on the blood and tissues of these animals.

Methods. Fed male rats weighing 200 to

[1] Noble, R. L., and Collip, J. B., *Quart. J. Exp. Physiol.*, 1942, **31**, 187.

[2] Noble, R. L., *Am. J. Physiol.*, 1943, **138**, 346.