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.

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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.

300 g were used in all cases except when heart glycogen was determined. Here females of 140 g were used. The animals were subjected to degrees of trauma varying from 300 to 700 turns of the drum—causing from 5% to 60% mortality in animals of this size. The resistant rats had received 1000 turns at weekly intervals over a period of several weeks. In most of the experiments, following trauma the animals were anaesthetized with nembutal, the hind leg muscles were frozen *in situ* in CO_2-ether mixture by the usual procedure, and the blood was taken by heart puncture. The liver samples were removed immediately after the bleeding. In experiments in which only sugar and lactic acid determinations were done, blood samples were taken repeatedly from the tails of unanesthetized animals.

The following determinations were made. Glycogen was done by the Good, Kramer, and Somogyi method,[3] and reducing and blood sugars by Somogyi's modification.[4] Lactic acid was done by a modified Barker and Summerson procedure,[5] the muscle first being thoroughly extracted with trichloracetic acid. Pyruvic acid was determined by Klein's procedure,[6] taking into account the recommendations of Elgart and Nelson.[7] Na and K were estimated by recent modifications of the zinc uranium acetate and the silver cobaltnitrite methods.[8] Amino acids were determined by Frame, Russell, and Wilhelm's modification of the Folin Method,[9] allowance being made for the uric acid present. NPN was done on tungstic acid filtrate by the micro-Kjeldahl distillation method, and the ammonia titrated with N/100 HCl.[10] Urea nitrogen was done according to the Folin and Wu method, using micro-Kjeldahl distillation and titration as above.[11] Uric acid was done colorimetrically by the method of Benedict and Behre[12] and creatinine and creatine by the method of Folin and Wu.[13] Phosphates were determined according to Fiske and Subbarow,[14] and serum calcium according to Clark and Collip's modification of the Tisdall method.[15]

The results shown in Tables I and II were analyzed statistically, expressed as the mean and standard error, and the significance of the changes determined. (Complete data available on request.) In the normal animal following trauma a significant increase was noted in blood sugar and pyruvic acid, and in blood and muscle lactic acid, the highest values being obtained in from 15 to 30 minutes after removal from the drum. The resistant animal after trauma showed an increase in all these constituents with the exception of pyruvic acid, but the differences in response between the normal and resistant groups were highly significant. The muscle and liver glycogen values reflected the changes seen in the blood sugar and lactic acid. At 6 hours, when the blood sugar was still slightly above normal (133 mg), the average liver glycogen was 0.91% in 6 animals, while the muscle glycogen tended to return to normal (365 mg %). Heart glycogens done on 4 animals showed no change following moderate or severe trauma. A small group of normal rats showed nearly 100% increase in blood phosphate, while in the resistant animals phosphate remained unchanged or tended to fall. A similar change occurred in serum phosphates. Ca remained practically unchanged. Serum Na showed no significant change in the normal or in the resistant rat, while K showed a slight rise in the normal at 15 minutes but remained unchanged throughout in the resistant animal. Haemolysis occurred in some animals following the trauma. This may be responsible for the rise in K in the normal rat. Clarke and Cleghorn, using the same method, report

[3] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

[4] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

[5] Barker, S. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, **138**, 535.

[6] Klein, D., *J. Biol. Chem.*, 1941, **137**, 311.

[7] Elgart, S., and Nelson, N., *J. Biol. Chem.*, 1941, **138**, 443.

[8] Neufeld, A. H., *J. Biol. Chem.*, in press.

[9] Frame, E. G., Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.*, 1943, **149**, 255.

[10] Peters, J. P., and van Slyke, D. D., 1932, *Quantitative Clinical Chemistry*, Baltimore, Vol. II. pp. 63 and 530.

[11] *Ibid.*, p. 557.

[12] *Ibid.*, p. 593.

[13] *Ibid.*, p. 604.

[14] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **55**, 375.

[15] Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

TABLE I.
Blood Analyses.

Blood (B) and Serum (S) Analyses, mg %		No. of turns in drum	Control	Minutes after removal from drum: 15	30	60-120
B. Sugar	N*	300	124 (11)†	140 (11)†	133 (10)†	117 (15)†
B. "	TR*	300	113 (6)	132 (6)	117 (5)	112 (6)
B. "	N	500-600	125 (22)	192 (17)	151 (9)	153 (14)
B. "	TR	500-600	128 (14)	141 (18)	141 (16)	138 (12)
B. Lactic Acid	N	300	36 (11)	135 (11)	56 (11)	42 (15)
B. " "	TR	300	32 (6)	67 (4)	42 (6)	36 (6)
B. " "	N	500-600	36 (17)	190 (21)	110 (14)	46 (8)
B. " "	TR	500-600	28 (8)	70 (10)	48 (10)	26 (8)
B. Pyruvic Acid	N	500	0.80 (6)	0.92 (6)	1.28 (6)	0.80 (6)
B. " "	TR	500	0.84 (6)	0.74 (4)	0.80 (6)	0.75 (4)
S. Sodium	N	500-600	211 (10)	208 (12)	218 (9)	209 (11)
S. "	TR	500-600	216 (6)	234 (6)	243 (6)	
S. Potassium	N	500-600	22 (10)	27 (8)	23 (11)	24 (10)
S. "	TR	500-600	20 (6)	19.8 (6)	19.8 (6)	
B. N.P.N.	N	500-700	38 (20)	56 (12)	60 (25)	63 (13)
B. "	TR	500-700	38 (8)	37 (6)	33 (8)	38 (4)
B. Urea N	N	500	14.7 (10)		21.6 (11)	
B. Uric Acid-N	N	500	0.82 (6)		1.48 (8)	
B. Creatinine-N	N	500	0.29 (10)		0.37 (11)	
B. Creatine-N	N	500	1.86 (10)		2.49 (11)	
B. Amino Acid N	N	500	9.4 (6)		13.4 (8)	
Rest N	N	500	5.4 (6)		10.2 (8)	
B. Phosphate	N	500	4.83 (3)	9.86 (3)	7.05 (3)	5.56 (3)
B. "	TR	500	4.35 (2)	4.40 (4)	3.70 (2)	3.80 (4)
S. "	N	500	5.95 (4)	10.92 (4)	9.97 (4)	
S. "	TR	500	5.92 (4)	6.15 (4)	5.57 (4)	
S. Calcium	N	500	10.2 (4)	10.77 (4)	10.87 (4)	

* N—Normal rats.
* TR—Rats made resistant to trauma as described in the text.
† No. of animals used.

TABLE II.
Tissue Analyses. 500-600 Turns of Drum.

Liver (L) and Muscle (M) Analyses, mg %		Control	Minutes after removal from drum: 15	30	60-120
L. Glycogen	N*	3520 (20)†	2610 (24)†	2280 (12)†	2220 (17)†
L. "	TR*	2410 (12)	3440 (9)	2690 (12)	1170 (3)
M. "	N	497 (14)	347 (9)	309 (14)	329 (10)
M. "	TR	529 (8)	443 (5)	477 (9)	
M. Lactic Acid	N	30 (6)	158 (10)	109 (6)	95 (4)
M. " "	TR	22 (6)	52 (4)	46 (6)	
M. Sodium	N	62 (13)	53 (11)	55 (5)	56 (11)
M. "	TR	57 (10)	43 (6)	54 (10)	
M. Potassium	N	353 (14)	364 (16)	377 (9)	340 (11)
M. "	TR	358 (10)	360 (6)	353 (10)	

* N—Normal rats.
* TR—Rats made resistant to trauma as described in the text.
† No. of animals used.

similar results.[16] There were no significant changes in either Na or K of muscle, although the Na showed a non-significant fall, and the K a rise in both groups. Non-protein N rose significantly in the normal at 15 minutes and remained elevated for at least 2 hours. As can be seen from the N partition in Table I, the increase in all the components was significant except in the case of creatinine. There was no increase in NPN in the resistant rat.

Amounts of blood withdrawn were found in control experiments not to be sufficient to

[16] Clarke, A. P. W., and Cleghorn, R. A., *Endocrinology*, 1942, **31**, 597.

produce any significant biochemical changes *per se*. Similarly, alterations in blood volume as determined by hemoglobin estimations were not related to these changes. Control experiments with adrenaline (0.25 mg per kg) produced the same elevation of the blood sugar in the resistant as in the normal animal.

The differences in blood and tissue chemistry shown by the normal and the resistant rat in response to trauma seem to indicate that the resistance which develops as a result of repeated traumatization, involved a stabilization of metabolic processes which are markedly upset in the non-resistant traumatized animal.

Summary. Biochemical changes in the blood and tissues of fed normal rats, and of rats made resistant to trauma, have been studied following the subjection of these animals to trauma in a revolving drum.

NPN rose significantly in the normal, but remained unchanged in the resistant rat.

Blood sugar, and blood and muscle lactic acid, rose in both the normal and resistant, but the difference between the two groups was highly significant. Pyruvic acid rose significantly in the normal, but showed no change in the resistant animal.

While whole blood and serum PO_4 rose 100% in the normal, Ca remained unchanged. There was no change in PO_4 in the resistant rat.

Values for Na and K of blood and muscle, and for liver, heart, and skeletal muscle glycogen showed non-significant differences between normal and resistant animals.

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Drug Prophylaxis against Lethal Effects of Severe Anoxia: VI. Neostigmine Bromide and Diphenylhydantoin.*

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In a preliminary survey of effects of autonomic agents on mortality of mice exposed to acute anoxic anoxia, it was noted that large doses of physostigmine and certain other cholinergic drugs tend to protect vs. anoxia while large doses of neostigmine methylsulfate do not do so and instead appear to be harmful. It was thought advisable to study this unexpected result in more detail.

Neostigmine bromide was administered intraperitoneally (ip.) or orally to 240 mice in doses listed in Table I, 30-60 min. before exposure to anoxia. The higher doses were within the lethal range: 5/20 mice treated with 0.3 mg/kg ip., 1/20 treated with 0.5 mg/kg orally, and 3/20 treated with 0.3 mg/kg ip. + diphenylhydantoin ip. died before exposure to anoxia.

Diphenylhydantoin Na was also administered to 140 mice, 110 of which had received neostigmine a few minutes before this treatment. All injections of dilantin were of 100 mg/kg ip. and were given approximately 30 minutes before exposure to anoxia. This agent has been shown by Hoff and Yahn[2] to be of value in protecting rats vs. acute anoxia and was used in the present study to test its effects in mice and also to determine whether or not a prophylactic synergy results from combination with neostigmine therapy.

Anoxia was induced by a standard method previously described.[3] Treated mice together with an equal number of control mice were exposed with adequate ventilation in a decompression chamber for 10 minutes to a reduced atmospheric pressure corresponding to an ap-

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1 Emerson, G. A., and Van Liere, E. J., *J. Lab. and Clin. Med.*, 1943, **28**, 700.

2 Hoff, E. C., and Yahn, C., *Federation Proc.*, 1943, **2**, 22.

3 Emerson, G. A., and Van Liere, E. J., *J. Lab. and Clin. Med.*, 1943, **28**, 689.