TABLE 1

EFFECT OF ESSENTIAL FATTY ACIDS ON LIPOTROPIC ACTION OF CHOLINE AND INOSITOL

Diet No.	Supplement per cent. of diet	Fatty acids per cent. liver weight
1. (Basal)	.....	23.4
2.	Choline chloride (0.5)	6.43
3.	Inositol (0.3)	13.3
4.	Mazola Oil (1.0)	25.3
5.	Mazola Oil (1.0) + Inositol (0.3)	27.2
6.	Mazola Oil (1.0) + Choline (0.5)	4.71

the other hand was obliterated by the inclusion of Mazola oil.[3] A possible explanation of this phenomenon might be that certain fatty acids in this oil make the diet more nearly adequate, increasing the demand for lipotropic factors and thus promoting a greater deposition of fat in the liver. But in view of the results with choline the writer believes that one must look elsewhere for the true explanation.

It is more probable that the nature of the fatty liver is changed in the presence or absence of the various supplements used. It will be recalled that choline has a relatively greater lipotropic effect on the "fat" fatty liver than on the "cholesterol" type of fatty liver, whereas with inositol, the reverse is true.[4] Only a complete analysis of the liver fats would reveal whether or not such a hypothesis is tenable. Fractionation of the fats from the livers of rats fed diets identical with those described above is now in progress and the results will be published shortly.

J. M. R. BEVERIDGE

BANTING AND BEST DEPARTMENT OF MEDICAL RESEARCH,
UNIVERSITY OF TORONTO

STUDIES ON THE TOXICITY AND ACTIVITY OF STREPTOTHRICIN

IN 1941 Waksman and Woodruff[1] reported on the isolation and properties of streptothricin, a bactericidal substance obtained from a soil organism named *A. lavendulae*. Recently, Foster and Woodruff[2] published on the *in vitro* action of streptothricin against bacteria, fungi and yeast. However, with the exception of a short note published by Metzger[3] on the action of this agent in experimental brucellosis, nothing has appeared in the literature regarding the *in vivo* activity or toxicity of this substance. The present communication is mainly concerned with these factors.

[3] Preliminary results of current investigations indicate that the substitution of a fat devoid of essential fatty acids does not interfere with the lipotropic action of inositol.

[4] G. Gavin, J. M. Patterson and E. W. McHenry, *Jour. Biol. Chem.*, 148: 275, 1943.

[1] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exp. Biol. and Med.*, 49: 207, 1942.

[2] J. W. Foster and H. B. Woodruff, *Arch. of Biochem.*, 3: 241, 1943.

[3] H. J. Metzger, S. A. Waksman and L. H. Pugh, *Proc. Soc. Exp. Biol. and Med.*, 51: 251, 1942.

EXPERIMENTAL

Materials: The streptothricin[4] used varied in potency from 5,000 to 300,000 units[5] per gram of solid. The drug is readily soluble in water, and was administered as an aqueous solution. The mice used were of the CFI strain and weighed between 18 to 21 grams each.

Toxicity Studies: These experiments were performed in mice by administering single doses of streptothricin intravenously, subcutaneously and by mouth. The dose levels employed and the data obtained are presented in summary form in Table 1. Mice injected intravenously or subcutaneously with dose levels of 30,000 units per kgm produced no evidence of toxicity throughout the five-day observation period. Dose levels of 60,000 units/kgm (approximately 10 to 12 times the effective dose) produce some deaths when given by vein, but no untoward effect by the subcutaneous or oral route. Large doses by the subcutaneous route produced toxic signs in mice. Streptothricin was well tolerated when given by mouth in that single doses of 250,000 units per kgm appeared

TABLE 1

ACUTE TOXICITY OF STREPTOTHRICIN FOR MICE

Dose in units/kgm	No. of mice/dose	Per cent. mortality (5 days observation) i.v.	s.c.	oral
30,000	10	0	0	0
60,000	10	20	0	0
125,000	10	20	30	0
250,000	10	80	100	0
500,000	10	100	100	10
750,000	10	...	...	30

TABLE 2

BACTERIOSTATIC ACTION OF STREPTOTHRICIN IN AGAR

Organism	Units per cc of agar required to produce complete inhibition
Strep. hemolyticus 1685	32
Strep. hemolyticus MIT	256
Strep. hemolyticus M	256
Strep. viridans	> 1024
Strep. lactis	> 1024
Staph. aureus SM	16
Staph. aureus FDA	128
Staph. aureus SD	128
Staph. aureus 155	128
Diplo. pneumoniae Type I	32
B. mycoides	1024
B. subtilis	32
E. typhi	4
S. aertrycke	16
S. enteritidis	64
S. schottmülleri	16
B. flexneri	32
B. sonne	128
P. lepiseptica	32
B. proteus	512
B. pyocyaneus	256
N. meningitidis	256
E. coli	16
S. leutea	256
A. aerogenes	256

[4] The streptothricin employed in these studies was obtained from the chemists of the Research Laboratories of Merck and Co., Inc., from cultures grown by Dr. J. W. Foster.

[5] A unit of streptothricin is the minimum quantity of drug which when added to 1.0 cc of nutrient broth will inhibit a given strain of *E. coli*.

to be without effect on mice. However, when sufficiently large doses (500,000 units/kgm) were given, signs of anorexia, accompanied by a gradual loss of weight, developed in most animals, and 10 per cent. of the mice died.

Efficacy: In vitro studies performed by incorporating streptothricin in melted blood agar and streaking the surface of the solidified agar with a variety of pathogenic bacteria, show this substance to be highly effective against organisms of both the gram-negative and gram-positive group (Table 2). Organisms of the colon-typhoid group and the Salmonella group are particularly sensitive. Thus quantities as small as 4 units per cc of agar were sufficient to inhibit completely the growth of *E. typhi*. Certain strains, such as *B. pyocyaneus* and *B. proteus, S. viridans* and *S. lactis,* were highly resistant to streptothricin.

The *in vivo* experiments were performed with a number of gram-negative and gram-positive pathogens. Infection was produced by intraperitoneal injection of 10,000 lethal doses of the test organisms, and treatment, initiated immediately after the infection, was given intravenously, intraperitoneally, subcutaneously and by mouth. Therapy consisted of either a single or repeated doses, the latter varying from once every six hours to once daily over a five-day period.

The results obtained with a number of the gram-negative organisms were of the same order, and therefore only the findings with a single test organism are presented in Table 3.

Streptothricin in single doses of 50 to 100 units per mouse (2500 to 5000 units/kgm) given intravenously or subcutaneously shortly after the bacterial inoculation afforded excellent protection against *S. schottmülleri* infections. Smaller amounts given intraperitoneally protected a large percentage of the mice. Similar results were obtained with strains of *S. aertrycke, E. coli and B. shigalis.* When administered by mouth, streptothricin was much less effective than following parenteral therapy. Doses of 3,000 units per mouse were required by mouth to afford the same protection as 100 units by vein or by the subcutaneous route. Repeated doses of streptothricin appeared to offer no great advantage over the single dose therapy, although in the lower dose levels the best results were obtained by administering the drug every six hours.

TABLE 3

EFFICACY OF STREPTOTHRICIN IN MICE INFECTED WITH S. SCHOTTMÜLLERI (SUBCUTANEOUS THERAPY)

Organism: *Salmonella schottmülleri.*
Age of Culture: 6 hours.
Infection: 0.5 cc of a 10^{-5} culture dilution in 4 per cent. mucin.
Therapy: Streptothricin given subcutaneously immediately after bacterial inoculation.

No. of mice	Drug	Units/dose	No. of doses/day	Culture dilution	No. surviving in days								Per cent. survival
					1	2	3	4	5	6	7	8	
(Therapy: A single dose)													
30	Strepto-	12.5	1	10^{-5}	3	0	0	0	0	0	0	0	0
65	thricin	25.0	1	"	25	23	18	16	15	15	15	14	21.5
65		50.0	1	"	49	44	43	41	37	37	34	34	52.4
65		100.0	1	"	65	62	62	60	59	59	59	59	90.8
35		200.0	1	"	35	35	35	35	35	35	35	35	100.0
(Therapy: Single Daily Doses over a 5-day period)													
20	Strepto-	12.5	1	10^{-5}	2	1	0	0	0	0	0	0	0
20	thricin	25.0	1	"	9	5	4	3	1	1	1	1	5
20		50.0	1	"	18	13	12	12	12	11	11	11	55
(Therapy: Every 6 hours over a 5-day period)													
20	Strepto-	12.5	4	10^{-5}	20	4	3	3	3	3	3	3	15
20	thricin	25.0	4	"	20	6	3	3	1	1	1	1	5
20		50.0	4	"	20	20	20	20	20	20	20	19	95
20		100.0	4	"	20	20	20	20	20	20	20	20	100
(Therapy: None)													
65	Controls	....	..	10^{-5}	6	1	0	0	0	0	0	0	0
30	"	....	..	10^{-6}	3	0	0	0	0	0	0	0	0
30	"	....	..	10^{-7}	16	10	7	5	3	3	3	3	10
30	"	....	..	10^{-8}	16	9	8	7	6	6	6	6	20

Certain gram-negative organisms and most gram-positive species were quite resistant to the action of streptothricin *in vivo.* The course of the infection in mice produced by strains of *B. pyocyaneus, B. proteus, Staph. aureus* and *D. pneumoniae* was not markedly influenced by streptothricin, even when doses approaching the toxic range were administered. Likewise, streptothricin had no significant influence on the virus of epidemic influenza or on *Trypanosoma equiperdum* infections in mice.

SUMMARY AND DISCUSSION

The foregoing experiments indicate that crude streptothricin is markedly effective *in vitro* against many gram-positive and gram-negative organisms. Furthermore, mice heavily infected with a variety of gram-negative organisms are completely protected by

the administration of small amounts of streptothricin. The drug is more effective parenterally than when given orally. However, preliminary experiments show that streptothricin by mouth greatly reduces the lactose fermenting bacteria of the intestinal tract. In this respect the drug is similar to certain sulfonamides and suggests, therefore, that streptothricin may be of value in bacillary dysentery and typhoid fever. The marked effect of streptothricin *in vitro* against gram-negative and gram-positive organisms, coupled with the fact that body fluids have no apparent inhibitory effect on the action of streptothricin, suggest that the crude drug might be of great value in infected wounds and burns.

HARRY J. ROBINSON
OTTO E. GRAESSLE
DOROTHY G. SMITH
MERCK INSTITUTE FOR THERAPEUTIC RESEARCH,
RAHWAY, N. J.

THE TIDAL AIR OF LABORATORY ANIMALS[1]

BECAUSE of the increasing need for information concerning the tidal air of laboratory animals we desire to present a general formula for such a determination on resting and fasting animals and to present the results of experimental tidal air determinations under conditions more nearly resembling those found during inhalation experiments with infectious nuclei.

In determining the tidal air by formula it must be assumed that animals use up the same proportion of oxygen from the air as man, *i.e.*, approximately 5 per cent. Thus, animals under basal conditions inspire 20 liters of air for each liter of oxygen consumed. The oxygen consumption in 24 hours in animals and man can be estimated from the basal heat production which for warm-blooded animals from rats to steers averages (72 $W^{3/4}$) calories, where W is the body weight in kilograms.[2] Since one liter of oxygen consumed by fasting animals represents 4.7 kilocalories of heat, the basal rate of oxygen consumption amounts to $\frac{72}{4.7} W^{3/4} = 15.3\ W^{3/4}$ liters of oxygen per day, or 306 $W^{3/4}$ liters of air per day, or $\frac{306 \times 1000}{1440} W^{3/4} = 212\ W^{3/4}$ cc of air per minute.

To determine the tidal air of 27-day-old albino Swiss mice under conditions more nearly approaching those found during inhalation experiments in which the mice are not at a basal condition, respiration trials were made in an apparatus previously described by Kleiber.[3] Animals were taken from a large cage containing food and water, divided into 7 groups of 10 mice each, and then placed in the respiration cages. The temperature during the run was 30° C. The mean metabolic rate of the animals during the whole trial (3 hours) was 119 kilo-cal./day/$Kg^{3/4}$; for the third hour only it was 97 kilo-cal./day/$Kg^{3/4}$. The mean weight per mouse at the end of the trial was 10.5 ± 0.25 gms. By the use of the formula, resting and fasting mice of 10.5 gms weight are calculated to have a tidal air of 7.0 cc per minute.

The tidal air of these animals as calculated from the metabolic rate was obviously decreasing from the start of the fast, as shown by the following figures.

Time in hours from start of fast	Mean tidal air per mouse per minute, in cc's
0.5	15.0 ± 0.8
1.0	11.7 ± 0.8
1.5	10.8 ± 0.7
2.0	10.5 ± 0.5
2.5	8.7 ± 0.3
3.0	9.3 ± 0.4

Some of the high rate in the initial half hour may be due to the effect of handling of the animals, but it is believed that the fasting was an important factor in the observed decrease. These data are comparable to the results of Loosli, Robertson and Puck[4] who used heavier, partially anesthetized animals and a different technique.

In some recently published experiments[5] we had occasion to determine the tidal air of a 3.5 kg *Macacus rhesus* monkey under intravenous pentabarbital anesthesia by means of a tracheal canula attached to a respirometer. Under deep anesthesia, when the respirations were shallow, three respirometer trials averaged 546 cc of air per minute. A resting and fasting monkey of the same weight would have a tidal air of 543 cc per minute as determined by the formula.

The basic heat formula is a useful tool for those interested in the biology of respiratory infections, since the tidal air of any laboratory animal can be quickly estimated if the weight is known. The data presented point out the need of having animals in a basal state in order to reduce the number of variables in inhalation experiments.

THE PERSONNEL OF U. S. NAVY
MEDICAL RESEARCH UNIT NO. 1
BERKELEY, CALIF.
MAX KLEIBER
COLLEGE OF AGRICULTURE,
UNIVERSITY OF CALIFERNIA, DAVIS

[1] The opinions advanced in this paper are those of the writers and do not represent the official views of the Navy Department.

[2] M. Kleiber, *Hilgardia*, 6: 315, 1931.

[3] M. Kleiber, *Univ. Calif. Pub. Physiol.*, 8: 207, 1940.

[4] C. G. Loosli, O. H. Robertson and T. T. Puck, *Jour. Inf. Dis.*, 72: 142, 1943.

[5] Personnel of U. S. N. Laboratory Research Unit Number 1 and W. R. Lyons, *Amer. Jour. Med Sci.*, 207: 40, 1944.