the first 6 to 12 days of the experiment, followed by mildly scaly paws and blood-stained noses and fur at 3 weeks. The fur coat of the animals in these 2 groups was generally poor from the first week and one case of extreme abdominal alopecia was noted at 5 weeks. The superiority of butter fat over corn oil (Table I, Groups 1 and 2) with respect to weight gain and increased food consumption was again confirmed. It is obvious that these relationships and values were practically unchanged by the removal of diacetyl from butter or by the addition of that compound to corn oil (Groups 3 and 4).

A ration identical to the one outlined above but containing dextrose as the sole carbohydrate in place of the lactose was fed with butter fat and corn oil. The results are included in Table I. With dextrose as the sole source of carbohydrate in the ration, no difference was found between the growth rates or food intake of rats fed butter fat compared with those fed corn oil. Comparison of the results of the dextrose experiment with those of the lactose experiment show that growth of the animals on the former regime was greater than that on the latter, the difference being very marked in the case of the corn oil groups.

Discussion. From these data we conclude that on the rations investigated a flavor factor such as diacetyl or any substance removed from butter fat by chromatographing as described has little effect on the food consumption and growth of the male albino rat. There are no data in the literature which indicate that by improving the taste or flavor of the diet the growth and food consumption of the rat have been increased. Off flavor and rancidity are known to have an adverse effect. On purified diets the true explanation for the superior growth-promoting value of one substance over another must lie in the existence of specific compounds in that superior nutrient which results in a more favorable physiological response such that the animal grows at a faster rate and hence consumes more ration. Paired feeding penalizes the superior food and ultimately obliterates the difference as measured by the growth rate and well-being of the experimental animal.

Summary. With *ad libitum* feeding and with lactose as the sole carbohydrate, the growth and appearance of albino male rats fed butter fat was superior to that of animals fed corn oil. Removal of the flavoring agents from butter fat by chromatographing or the addition of one such agent, diacetyl, to corn oil had little effect on the comparative nutritive value of the two fats. Rats fed butter fat or corn oil on rations containing dextrose as the sole carbohydrate grew at equal rates and were of normal appearance. Flavor played no part in these dextrose experiments.

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Therapeutic Effects of Forbisen and of Toluidine Blue on Experimental Typhus.

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The therapy of rickettsial infections is almost entirely symptomatic. Various sulfonamides[1] have been used to treat patients, but their usefulness appears to be limited to the treatment of pneumonias or other secondary bacterial infections in the late stages of the disease. Neoarsphenamine in metaphen,[2] atebrin-plasmochin,[3] and atebrin-calcium[4]

[1] Wohlrab, V. W., *Klin. Wchnschr.*, 1942, **31**, 445.

[2] Baker, G. E., *Ann. Int. Med.*, 1942, **17**, 247.

[3] van Meerendonk, Piet, *Deut. Militärarzt.*, 1942, **7**, 283. Abstracted by Megaw, J. W. D. M., *Trop. Dis. Bull.*, 1942, **39**, 679.

mixtures have been employed in therapy, and favorable results have been reported. Further work will be required to assess their value accurately.

In the present study a large number of compounds were investigated for therapeutic activity in mice infected with murine typhus. Murine typhus infections in mice have been described recently by Gildermeister and Haagen[5] and Pang and Zia.[6] Yolk sacs of developing eggs infected by the Cox[7] technic contain sufficiently large numbers of rickettsiæ to kill mice by a toxic action within 2 to 8 hours after intraperitoneal injection.[5] The dose, however, can be adjusted so that the mice are not affected by the toxin but die 4 to 5 days later of rickettsial infection. Mice are as susceptible to the toxin of epidemic strains as that of the murine typhus, but they are completely resistant to infection by epidemic strains.

Materials and Methods. Pools of typhus-infected yolk sac material diluted to a final concentration of 20% in broth were stored in flame-sealed ampoules in the CO_2 icebox. Single ampoules containing 5 cc of material were removed for each experiment. The murine-infected yolk sac suspension diluted to a final concentration of 0.33 to 1.2% usually killed 80 to 100% of the control mice when injected intraperitoneally in 0.5 cc quantity. Compounds being tested were fed in a 1% concentration in finely ground fox chow. No other food was given. Water was supplied by water bottles. The mice were kept in glass jars in groups of 5. Twenty mice were usually used in testing each compound, and a similar group of controls was infected. The feeding of each test drug was started 4 to 24 hours before the infection was given. It was hoped that slight degrees of activity would be detected by this procedure. Several animals dying in the treated and control groups were autopsied, and smears of peritoneal fluid made and examined microscopically to verify the presence of rickettsia. The Wilmington strain of murine typhus was used throughout except for a few experiments with the Breinl epidemic strain. The results are given below.

Forbisen. Forbisen* was found to possess moderate activity. It was necessary to feed it in a concentration of 2.5% in the diet to produce a significant recovery rate among the treated mice. In a typical experiment 14 of 24 infected mice survived when fed 2.5% forbisen. All of 20 control mice died of the infection.

Toluidine blue. Toluidine blue was very efficacious in preventing deaths from typhus. In an early experiment 40 mice were fed fox chow containing 1.5% toluidine blue. These, along with 20 control mice, were infected 24 hours after feeding was begun. Two treated mice were killed on the 4th day. Of the remaining 38 mice, 27 recovered. There were 4 survivors in the group of 20 control animals. The animals treated with toluidine blue usually showed few or no signs of illness. Deaths usually occurred later than in the untreated group. In the above-described experiment half of the control mice died on or before the 4th day of infection. Three treated mice died in the same interval. The optimum dosage of toluidine blue appeared to be supplied when concentrations of 0.25 to 0.75% were fed in the diet. This represented an ingestion of about 5 to 20 mg a day per mouse. Larger or smaller doses in the diet were often accompanied by an increased mortality. Therapeutic activity could be demonstrated when toluidine blue was administered as late as 24 hours after the infection.

Toluidine blue has a marked action against the Wilmington murine strain *in vitro*. It was added to 5 different concentrations of infected yolk sac suspension to make a final concentration of 1:10,000 toluidine blue. The same quantity of distilled water was added to the infected yolk sac suspensions used as controls. Both sets of tubes were placed in ice water

[4] van Meerendonk, Piet, *Deut. Militärarzt.*, 1942, **7**, 541. Abstracted by Megaw, J. W. D. M., *Trop. Dis. Bull.*, 1943, **40**, 237.

[5] Gildermeister, E., and Haagen, E., *Deutsche Med. Wchnschr.*, 1940, **66**, 878.

[6] Pang, K. H., and Zia, S. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 76.

[7] Cox, H. R., *Pub. Health Reports, U.S.P.H.S.*, 1938, **53**, 2241; 1940, **55**, 110.

* Supplied by Mr. W. W. Allen of the Dow Chemical Company.

TABLE I.
Effect of Administering Toluidine Blue at Various Times before and after Infecting Mice with Typhus.

	Feeding of 0.5% toluidine blue in diet started					
	24 hr prior to infection	At time of infection	24 hr after infection	48 hr after infection*	72 hr after infection	Control
No. mice infected	19	20	20	20	20	20
Survivors	14	15	10	1	4	2
% surviving	74	75	50	5	20	10

* Some mice in this group had died and the rest were sick by the time toluidine blue administration was started. Feeding started at 48 hours or later resulted in ingestion of insignificant quantities of toluidine blue.

TABLE II.
The *in vitro* Effect of Toluidine Blue on Rickettsial Toxicity and Infectiousness for Mice.*

% conc. yolk sac, 0.2 cc injected i.v.	Mice surviving injection			
	Murine strain in ice water 1 hr		Epidemic strain incubated 37°C 1 hr	
	Control	Toluidine blue 1:10,000	Control	Toluidine blue 1:10,000
10	0/6*	0/6		
5	0/6	1 (1)/6	0/6	0/6
2.5	0/6	6 (3)/6	0/6	6/6
1.25	1 (1)/6	6 (1)/6	5/6	6/6
0.62	6 (6)/6	6/6	6/6	6/6
Totals:				
No. injected	30	30	24	24
Survived toxin	7	19	11	18
Survived toxin and infection	0	14		

* Open numbers in numerator represent mice surviving toxin. Numbers in numerator in parentheses represent mice surviving toxin but dying of infection. Denominator equals number injected.

for 1 hour before their contents were injected intravenously into mice (Table II). There was a reduction in both the toxin and the infectious titer of the rickettsial suspensions with added toluidine blue. Similar treatment of suspensions of yolk sac infected with the Breinl epidemic strain resulted in no decrease in toxicity. If the contact between the rickettsia of this strain and the toluidine blue in ice water was prolonged to 3 hours, or if the mixture was incubated at 37°C for 1 hour, some reduction in the amount of toxin resulted.

Cotton rats[8] infected with either the murine or the epidemic strain of typhus by intracardial inoculation have been treated with toluidine blue as well as with forbisen. No evidence of significant therapeutic activity was obtained.

Discussion. The therapeutic efficiency of toluidine blue in treating typhus-infected mice was in marked contrast with the poor results obtained when cotton rats were treated. The latter animals ate irregularly and mostly at night. This may have made the dosage of any drug fed in the diet quite erratic and thus have contributed to the poor results. No attempt to determine the level of toluidine blue in blood was made. It is possible that the rapid dissemination of the organisms following intracardial inoculation of rickettsia may have overwhelmed the therapeutic activity of toluidine blue.

Summary. Two compounds, toluidine blue and forbisen, have been found to possess therapeutic action against murine typhus infections in mice.

[8] Snyder, J. C., and Anderson, C. R., *Science*, 1942, **95**, 23.