

tematic investigation with varying amounts of galactose in the diet might throw some more light on this question.

Summary. Chicks fed a diet containing a high percentage of galactose develop violent spasms and die within few days. There is a

high content of galactose in the blood during the spasms but about normal blood glucose. Muscle glycogen is about normal but liver glycogen nearly zero. The mechanism of the disorder is not yet clear.

14457

Use of a Germicidal Quaternary Ammonium Salt in Nutritional Studies.

L. J. TEPLY AND C. A. ELVEHJEM.

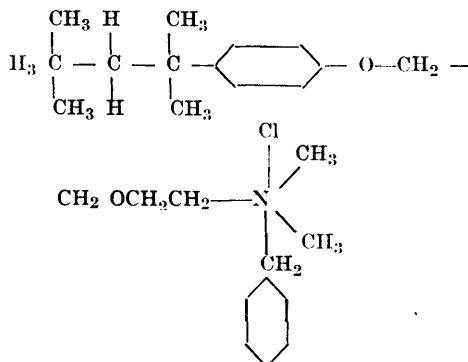
From the Department of Biochemistry, College of Agriculture, University of Wisconsin, Madison.

Until sterile rats can be raised routinely, studies involving synthesis of vitamins by bacteria existing in this animal will have to depend largely on the use of suitable agents to eliminate at least some of the microorganisms. Certain sulfa drugs have proven to be of much value in such studies, but it is recog-

the effect of feeding one of the quaternary ammonium salt germicides to rats on various diets.

Experimental. The compound Phemerol* was chosen because of its potency, purity, low toxicity, and availability.^{3,4} In all the studies, 21-day-old, male, albino Sprague-Dawley rats were used. The basal ration consisted of sucrose 76, Labco casein 18, salts IV⁵ 4, corn oil 2; vitamins (mg per 100 g ration): choline 100, nicotinic acid 0.250, calcium pantothenate 2.0, thiamine 0.200, pyridoxine 0.250, riboflavin 0.300, 2-methyl-1,4-naphthaquinone 0.100, biotin 0.010 (S.M.A. conc.) Two drops of haliver oil containing 1 mg α -tocopherol were fed weekly to each rat. When used, para-aminobenzoic acid and inositol were added at a level of 25 mg per 100 g of ration and vitamin C at 100 mg %. Three rats were used per group and growth results given in Table I are averages over a 5-week period.

It was found that when crystalline Phemerol was incorporated in the diet at a level as low as 0.1%, rats refused to eat the ration and soon died from inanition. A ration containing



nized that their usefulness is limited because they attack only certain types of bacteria while others survive and indeed tend to fill in the "vacancy" created by removal of the sulfonamide-susceptible types.^{1,2} It seems logical therefore that each new germicide whose properties suggest that it might be useful in nutritional studies should be given a trial. This report summarizes preliminary observations on

¹ Light, R., Cracas, L., Oliott, C., and Frey, C., *J. Nutr.*, 1942, **24**, 427.

² Gant, O., Ransone, B., McCoy, E., and Elvehjem, C., *Proc. Soc. Exp. Biol. and Med.*, 1943, **52**, 276.

* The Phemerol used in these studies was supplied by Parke, Davis and Co., Detroit, Mich.

³ Rawlins, A., Sweet, L., and Joslyn, D., *J. Am. Pharm. Assn.*, 1943, **22**, 11.

⁴ Joslyn, D., You, K., and Rawlins, A., *J. Am. Pharm. Assn.*, 1943, **22**, 49.

⁵ Hegsted, D., Mills, R., Elvehjem, C. A., and Hart, E., *J. Biol. Chem.*, 1941, **138**, 459.

TABLE I.
Growth of Rats Receiving Phemerol on Various Diets.

	Avg weekly gains in g	
	Series 1	Series 2
Basal	29	26
" + Phemerol	10 (1 dead)	11 (2 dead)
" + " + 5% Whole Liver Substance	23	26
" + " + 5% Dried Yeast	18	
" + " + 5% Grass Extr. Powder	20	
" + " + 5% Solubilized Liver Extr.	20	17
" + " + 2% " " " + P.A.B.	15	17
+ Inositol + Vit. C		21
Basal + Phemerol + Folic Acid $\approx$ 2% Solubilized Liver Extr.	7 (1 dead)	
" + " + " $\approx$ 4% " " " " "		
+ P.A.B. + Inositol	8	

this amount of Phemerol is irritating to the throat. However, the animals readily drank a 1-1500 water solution of Phemerol as their sole source of liquid and soon became accustomed to a 1-1000 solution. Therefore, in all subsequent experiments the drug was administered in the drinking water.

Twenty-one-day-old rats usually will not survive on a 1-1000 solution of Phemerol even though they are on a diet containing 5% whole liver powder. They usually survive on a 1-1200 solution but early growth is poor. We have found it desirable to start with a 1-2000 solution and gradually work up to a 1-1200 concentration within 2 weeks. This procedure was followed in experiments reported in Table I.

Because it was feared that lipoidal material might interfere with the action of Phemerol, in the early experiments the corn oil was fed by dropper, 3 drops every third day. However, we have found that similar results are obtained if the fat is mixed in the ration and this procedure was followed in series 2.

Preliminary work involving more than a hundred rats demonstrated the following facts: (1) If a 1-1500 Phemerol solution was used as the sole source of liquid by rats on the basal diet outlined above without biotin, poor growth was obtained, but if 5% whole liver powder was added, excellent growth resulted. (2) On a synthetic ration, a 1-2000 Phemerol solution had no effect over a period of several weeks. (3) On a 1-1500 solution with no biotin in the ration, addition of 2% solubilized liver extract only partially restored

the growth rate. Further addition of biotin produced some growth response and finally addition of 5% whole liver powder produced normal growth. All subsequent experiments were made with biotin in the ration.

Discussion. Results reported in Table I indicate that a combination of folic acid, para-aminobenzoic acid and inositol is not able to counteract the effects of Phemerol. Solubilized liver extract, yeast, yeast extract, and grass juice powder all partially counteract the effects of the drug at the levels fed. At equivalent levels, whole liver powder seems to be superior to solubilized liver extract, grass juice powder or dried yeast.

These results are quite different from those obtained with sulfa drugs, in which case biotin and folic acid or solubilized liver extract seem to restore the growth rate very effectively. This, of course, might be expected if Phemerol attacked different types of bacteria than did the sulfa drugs.

Since it is possible that Phemerol is inactivated by intestinal contents or is absorbed in the first portion of the G-I tract, it is interesting to consider its possible effects in the mouth, throat, and stomach. A bacterial cell which grows in the mouth, synthesizes vitamins, and is then digested and releases these vitamins would certainly be of greater nutritional significance than a large number of bacteria in the intestines which synthesized vitamins but retained them within the cells and passed out in the feces. Sherman *et al.*⁶

⁶ Sherman, J., Niven, C., and Smiley, K., *J. Bact.*, 1943, **45**, 249.

have demonstrated that *Streptococcus salivarius*, which requires only 5 vitamins on a synthetic medium, occurs in large numbers in the mouths and throats of normal individuals and in some cases thrives in the intestines.

Summary. Administration of Phemerol to

rats in the drinking water at a 1-1500 or 1-1200 dilution results in poor growth on a synthetic ration. Addition of 5% whole liver powder gives good growth. A combination of folic acid, para-aminobenzoic acid, and inositol does not counteract the effects of the drug.

14458

Alterations in Mammary Structure Following Adrenalectomy in the Immature Male Rat.*

CHARLES F. REEDER AND S. L. LEONARD.

From the Department of Zoology, Cornell University, Ithaca, N.Y.

It has been reported that adrenalectomy in underfed intact and castrated female rats caused the mammary glands to grow more rapidly, to increase the number of bud-like growths along the course and at the ends of the ducts, and to increase the area covered by the gland.¹ In adrenalectomized lactating rats, the mammary alveoli were reported less distended than normally but were restored by NaCl therapy.² The paucity of data on the structural changes in the mammary gland subsequent to adrenal ablation suggested further investigations.

Young male rats of the Long-Evans and Sprague-Dawley strains were used, the ages ranging from 22 to 35 days at the time of adrenalectomy. An adequate stock diet plus tap water and a 1% NaCl solution was fed *ad libitum*. Bilateral adrenalectomy was performed in one operation and in experiments in which castrated males were used, the latter operation was carried out the preceding day. Autopsies were made 10-12 days later.

In some experiments, normal and castrated adrenalectomized rats, together with their controls, received 5 μ g of alpha estradiol dipropionate (Di-Ovocylin)[†] daily for 10 days.

* Aided by a grant from the Sackett Fund of the Trustee-Faculty Committee on Research.

¹ Butcher, E. O., *Proc. Soc. Exp. Biol. and Med.*, 1939, **42**, 571.

² Levinstein, Irving, *Anat. Rec.*, 1937, **67**, 477.

[†] Acknowledgment is made to Dr. E. Oppenheimer of Ciba Pharmaceutical Products, Inc., Summit, N.J., for a generous supply of hormone.

The right abdominal mammary glands were prepared as stained whole mounts for study.

Results. The most notable change in the mammary glands which followed adrenalectomy was an increase in the number of lateral buds or short branches along the duct system. This was seen in 14 of 17 young operated males compared to 9 controls. Half of the operated rats also showed an increased number of end buds, many of which were much enlarged. The gain in body weight of the adrenalectomized males averaged 14 g less than their controls but they were otherwise in an apparently good state of health. The greatest amount of gland development occurred in those operated animals which gained the most weight. Similarly, the glands in 6 of 10 adrenalectomized castrated males also showed a greater number of lateral duct buds when compared to those from 8 castrated controls. End buds were not observed to be stimulated. The areas of gland tissue in the adrenalectomized animals in both groups were not more extensive than in the controls.

When estrogen treatment was given to 5 adrenalectomized and 4 control male rats, the increased number of lateral buds in the experimental group showed a marked dilatation. The average body weight gain in the adrenalectomized injected group was only half that of the controls. As before, the degree of lateral bud proliferation varied directly with the amount of weight gained.

Similarly, if castrated animals were used, and treated with estrogen, the mammary