eralization with respect to this mode of administration.

Pseudomonas pyrogen was used in only 2 dose ranges of 10-50 μg in 29 rats and 100-500 μg in 11 rats. Critical examination of the individual protocols indicates that this pyrogen had slightly less inhibitory effect on ulceration than the prodigiosus pyrogen which correlated with the comparatively low pyrogenic activity as measured in the rabbit test.

Typhoid pyrogen was used only in the larger dose range, two of eight rats showing minor ulceration with this preparation.

Not shown in the table but deserving of mention are results with a non-pyrogenic polysaccharide prepared by the pyrogen isolation technic from pneumococcus type II cultures. This substance showed no anti-ulcer activity in 4 rats in doses up to 200 μg.

Also omitted are detailed data on gastric volume and free acidity. However, it may be stated that among all rats studied in these and related experiments, the mean gastric volume of 208 ulcerated rats was 8.9 $\pm$ 2.6 (S.D.) ml whereas in 94 non-ulcerated rats secretion amounted to 5.9 $\pm$ 3.2 ml. Likewise free acidity of gastric contents from ulcerated rats was equivalent to 5.3 $\pm$ 2.4 ml N/10 NaOH. Non-ulcerated rats showed values of 3.0 $\pm$ 2.3 ml. Among both groups of data the differences between the means from ulcerated and non-ulcerated rats are statistically significant.

Conclusion. As judged by response in the Shay rat, pyrogens from *B. prodigiosus, Pseudomonas aerugenosa* and *E. typhi* are highly active ulcer-inhibiting substances. Whether the presence of these substances in various tissue and urinary extracts may account for the reported anti-ulcer activity of these preparations remains to be investigated. The mode of action of pyrogens is not clear but may be associated with the obviously diminished acidity and volume of gastric secretion which occurs as a result of administration of these substances. There was observed no evidence, however, of any general "toxicity" in the animals which received purified pyrogens.

Summary. Intravenously administered purified pyrogens from cultures of *B. prodigiosus, Pseudomonas aeruginosa* and *E. typhi* possessed marked ulcer-inhibiting action in the Shay rat.

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Increased Requirement for Pteroyl Glutamic Acid During Lactation.*

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A series of experiments had been undertaken in an attempt to produce an anemia in fetal and newborn rats. One of the technics employed was the feeding of succinyl sulfathiazole (SS) to the maternal rats. During the course of the study, it was observed that the newborn rats from mothers receiving the diet containing SS, died within five days after birth. It was thought that this high mortality among the newborn might be due to either a toxic effect or to a failure of lactation.

Preliminary experiments indicated that SS or its hydrolysis products did not have any toxic effect on the newborn rats. Although SS passes through the alimentary tract substantially unchanged and is not absorbed, some small part of it is hydrolyzed to sulfathiazole which may be absorbed. On a diet containing 1% SS, rats were found to have a blood level of approximately 0.3 mg % sulfathiazole.[1] The administration of SS or

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TABLE I.
Effect of Diet of Pregnant Rats on the Survival Time of the Newborn.

Diet	No. of litters	No. of newborn	Birth wt, g	Average survival, days	No. of newborn surviving 20 days	% mortality
Control	4	30	5.5	18.1	26	13
SS diet	12	94	5.3	2.2	0	100
SS " + liver	5	40	5.1	4.1	0	100
SS " + alfalfa	4	32	5.8	15.2	23	28

TABLE II.
Survival of Newborn Rats from Mothers on a High Pteroylglutamic Acid Diet.

Diet	No. of litters	No. of newborn	Birth wt, g	% of newborn surviving 20 days
Control	6	42	5.1	97
SS diet	8	56	5.2	91
SS " + alfalfa	8	55	5.2	96

sulfathiazole intragastrically, or intraperitoneally, at a level estimated to be about 10 times that ingested in the milk was without effect on the newborn rats from mothers on a normal diet. When small amounts of sulfathiazole (0.08%) were included in the diet, there was no effect on lactation. Since this dietary level of sulfathiazole produced about the same level of blood sulfathiazole as did 1% SS, the lactational failure could not be ascribed to a direct toxicity of SS or sulfathiazole.

A further experiment indicated that the fatality among the newborn rats from mothers receiving the diet containing SS was most probably the result of inanition. When newborn rats (12-36 hours after birth) from mothers on a normal diet were substituted for the newborn litter of a mother on a diet containing 1% SS, the normal newborn died within a few days. On the other hand, the newborn from mothers on the SS diet were successfully raised to weaning by mothers receiving a normal diet.

Since a toxic factor did not appear to be responsible, it was considered that the failure in lactation might be due to the need for some essential nutrient, normally synthesized by the intestinal flora, but now lacking because of bacterial suppression by the SS.

Experimental. In all the following experiments, female rats were placed on the control or experimental diets 10 days before mating. The females were kept on their respective diets until 20 days after the birth of their young. In the first series of experiments (Table I), the litters were limited to 8 young; in the second (Table II), to 7 young.

The control diet consisted of 28 g vitamin-free casein (SMACo), 56 g carbohydrate (sucrose or cerelose), 10 g lard, 2 g corn oil, 4 g salt mixture,[2] 1.0 mg thiamine HCl, 1.5 mg riboflavin, 1.0 mg pyridoxine HCl, 10 mg nicotinic acid amide, 4.0 mg calcium pantothenate, 100 mg inositol, 100 mg choline chloride, 100 mg p. aminobenzoic acid, 0.5 mg 2-methyl naphthoquinone, 0.05 mg biotin, 0.02 mg pteroylglutamic acid,† 2.0 mg α-tocopherol, 2500 IU vitamin A, and 360 IU vitamin D. The experimental diet (SS diet) was the same except that 1 g of succinyl sulfathiazole was substituted for an equal amount of carbohydrate. The diets were fed *ad libitum*.

A comparison was made of the effect of the control and SS diets on lactation. The survival time of the newborn rats was taken as a measure of lactation. Although some of the litters of newborn from the SS diet group had milk in their stomachs the first and second days, they were all dead by the fifth day

[1] Williamson, M. B., Abstr., *Div. Biol. Chem., Am. Chem. Soc.*, 1948, 55C.

[2] Hubbel, R., Mendel, L., and Wakeman, D., *J. Nutrition*, 1937, **14**, 237.

† The pteroylglutamic acid was generously supplied by Dr. M. C. Lockhart of the Lederle Laboratories.

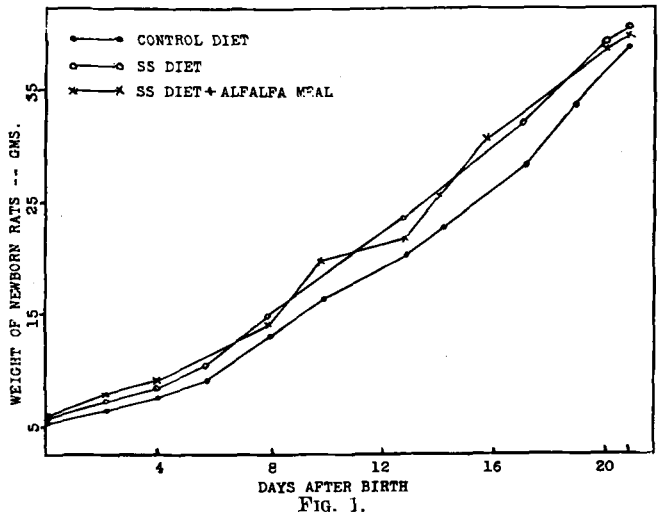

FIG. 1.

The rate of growth of newborn rats from mothers on a synthetic diet containing pteroylglutamic acid.

after birth; in no case was milk observed after the second day. This might be taken to indicate that in some cases, the crucial nutritional factor required to maintain lactation was not depleted until after one or two days of lactation. After the depletion, lactation failed with the consequent death of the newborn rats. Only 13% of the control newborn died before 20 days; it seems highly improbable that this mortality was due to lactational failure.

Further experiments in which supplements were added to the SS diet were undertaken. When 1% dry defatted liver powder was added to the SS diet, a slight but questionable increase in survival time was noted. In another experiment, 10% alfalfa meal was used. The casein content of the SS diet containing alfalfa meal was adjusted so that the total nitrogen content was the same as that of the control diet. The alfalfa meal supplement markedly improved the SS diet so that it was almost as efficient as the control diet in supporting lactation. The results of these experiments are shown in Table I.

Since it is known that added nutritional "stress" occurs during periods of pregnancy and lactation,[3] it was thought that there might possibly be some nutrient which was required at an even higher level than was supplied by the preceding diets. Richardson and Hogan[4] have reported the successful rearing of rat litters by mothers on a diet essentially the same as the control diet, except that it contained no pteroylglutamic acid or SS. The presence of SS in the diet, however, makes the necessity for exogenous pteroylglutamic acid crucial.[5] Therefore, the effect of a higher level of pteroylglutamic acid on lactation was investigated.

The control diet, the SS diet and the SS + alfalfa meal diet were supplemented to contain 1.0 mg of pteroylglutamic acid per 100 g of diet. Almost all the newborn rats from all groups survived until 20 days after birth. The results are indicated in Table II. The rates of growth of the newborn are shown in Fig. 1.

It can be seen that all the newborn grew at approximately the same rate, and hence, most likely received about the same amount of milk. It then follows that the level of pteroylglutamic acid in the maternal diet was at, or above, the optimal level for the support of lactation. Recent reports have shown the beneficial effects of pteroylglutamic acid on lactation.[6,7]

[3] Ershoff, B. H., *Physiol. Rev.*, 1948, **28**, 107.

[4] Spicer, S. S., Daft, F. S., Sebrell, W. H., and Ashburn, L. L., *Pub. Health Rep., U.S.P.H.S.*, 1942, **57**, 1559.

[5] Richardson, L. R., and Hogan, A. G., *J. Nutrition*, 1946, **32**, 459.

It is interesting to note that the maternal requirement for pteroylglutamic acid appears to be very much greater during the period of lactation than during pregnancy. This is supported by the fact that the birth weights of all the newborn were found to be about the same (5.5 ± 0.8 g) regardless of the maternal intake of pteroylglutamic acid.

Summary. The inclusion of succinyl sulfathiazole in the diet of maternal rats during pregnancy depresses lactation. Relatively high levels of pteroylglutamic acid are required to maintain lactation. Higher levels of pteroylglutamic acid appear to be required during lactation than are required during pregnancy.

[6] Nelson, M. M., and Evans, H. M., *Arch. Biochem.*, 1947, **13**, 265; 1948, **18**, 153.

[7] Sica, A. J., Allgeier, A. M., and Cerecedo, L. R., *Arch. Biochem.*, 1948, **18**, 119.

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Acetone-Ether Extracted Antigens for Complement Fixation with Certain Neurotropic Viruses.

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A method is described for the preparation of antigens for complement-fixation tests with certain neurotropic viruses. This antigen has been found to be practical, nonanticomplementary, specific and reliable. It is furthermore, easily prepared with ordinary laboratory equipment and can be made available, if necessary, within 6 hours from the time when the tissue is harvested, thus being useful for field work. Since the preparation depends on the elimination of the acetone-ether soluble fraction of the brain tissue with considerable reduction of lipid, it has the advantage of doing away with nonspecific reactions with Wassermann-positive human sera.[1,2]

Method of preparation. Brain tissue from mice infected intracerebrally with the desired virus is harvested; preliminary bleeding of the mice[2] is advisable in order to reduce the amount of hemoglobin present in the final product. The weighed tissue with 20 volumes of acetone is placed in a Waring blendor and allowed to run for 2 minutes. The blendor used for this purpose is surrounded by a metallic jacket which is filled with ice and water to maintain a low temperature; less preferably, the extraction can be carried out in the absence of this device. The suspension is then poured into a 250 ml bottle and centrifuged at 1500 rpm for 1 minute; the supernate is discarded. All the foregoing procedures should be rapidly performed. To the sediment is added 20 volumes (referred to the initial weight of the wet-brain tissue) of fresh acetone; the bottle is closed with a cork stopper, shaken by hand at intervals and kept at room temperature for 20 minutes. The preparation is then centrifuged as before, the supernate which is slightly opalescent, as are the subsequent supernates, is discarded. To the sediment is added 20 volumes of a mixture of equal parts of acetone and anhydrous ethyl ether. Again the suspension is kept at room temperature for 20 minutes, shaken occasionally by hand, centrifuged at 1500 rpm for 1 minute and the supernate discarded. The sediment is washed twice in succession with 20 volumes of ethyl ether each time and held for 20 minutes at room temperature. After the last ether extraction and centrifugation, the sediment is dried by connecting the centrifuge bottle with a vacuum oil pump; in a short time, from 15 minutes to 1 hour depending on the amount involved, the residual ether is evaporated and the sediment remains as a

[1] DeBoer, C. J., and Cox, H. R., *J. Bact.*, 1946, **51**, 613; *J. Immunol.*, 1947, **55**, 193.

[2] Espana, C., and Hammon, W. D., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 101.