

weighed 950 g when the supplement was added and at the time they started laying again their weight had increased to approximately 2 kg each.

The basal ration used in the second experiment was identical to that used in the first except that 0.1% inositol was added.

In this experiment 4 birds were fed the basal diet. Their average hatchability dropped from above 90% to less than 20% in 6 weeks. They were continued on the basal diet for 6 weeks longer to insure thorough depletion and then were fed the norite eluate fraction preparation equivalent to 10% solubilized liver extract. The results are given in Fig. 1.

The results of this experiment are in agreement with the results of the first experiment and show conclusively that the

norite eluate of solubilized liver extract contains a factor or factors essential for normal development of the chicken embryo.

A deficiency of this factor resulted in a less pronounced decline in egg production than in hatchability. Some birds continued to lay when fed the basal ration while others would go out of production and lose weight. Such results suggest that the basal ration contained a suboptimal amount of the factor involved. Further studies are in progress to clarify this point.

Summary. Evidence is presented to show that the *Lactobacillus casei* ϵ eluate fraction is essential for normal development of the chicken embryo.

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Chemotherapy in *Cl. welchii* Infection in Mice.

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The importance of *Cl. welchii* infection in war wounds has given impetus to work in this field during the past year. The early literature on the subject has been well reviewed by Bliss, Long and Smith.¹ Recently, Sewell, Dowdy and Vincent² have been able to produce an infection in dogs which closely simulated human infection. They found that sulfadiazine therapy gave the best results.

In this study, a comparison of the efficacy of sulfanilamide, sulfapyridine, sulfathiazole and sulfadiazine† in experimental *Cl. welchii* infection in mice has been made. By feeding

the animals a moist cooked diet in which the drugs had been incorporated, it was possible to maintain blood concentrations comparable to those used in the treatment of patients ill with serious infections.

The drug-diet was prepared in the following manner: The required amount of drug was dissolved in 1000 ml of water at 100°C. To this was added slowly 280 g of a modified Sherman dry diet and the mixture cooked with stirring until it was the consistency of thick mush. It was then allowed to cool, weighed and the amount of drug calculated as mg per g of food. The per cent of drug

* A. B. Kuppenheimer Foundation.

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¹ Bliss, E. A., Long, P. H., and Smith, D., *War Med.*, 1941, **1**, 799.

² Sewell, R. L., Dowdy, A. H., and Vincent, J. G., *Surg., Gyn. and Obst.*, 1942, **74**, 361.

‡ The sulfonamides used in this study were furnished through the courtesy of Lederle Laboratories, Inc. (sulfadiazine), Merck and Company (sulfapyridine), and the Department of Medical Research, Winthrop Chemical Company (sulfanilamide and sulfathiazole).

TABLE I.
Comparison of Drug Ingested and Blood Concentrations.

Drug-diet	Drug ingested		Blood concentrations mg/100 Mean $\pm$ S.E.
	Mg/mouse/day Mean $\pm$ S.E.	G/kg/day Mean $\pm$ S.E.	
2.0% sulfanilamide	41.6 $\pm$ 2.68	2.18 $\pm$.26	16.0 $\pm$ 1.15
1.5% sulfapyridine	36.1 $\pm$ 2.20	1.99 $\pm$.16	12.5 $\pm$ 2.04
2.0% sulfathiazole	37.6 $\pm$ 2.84	1.89 $\pm$.05	11.0 $\pm$ 0.82
0.4% sulfadiazine	11.1 $\pm$ 0.74	0.61 $\pm$.03	12.5 $\pm$ 1.48

used was calculated on the basis of the dry meal, *i.e.*, for a 1% drug-diet 2.8 g of drug was used, for a 2% diet 5.6 g, etc. The amounts correspond to roughly 0.2 and 0.4% concentrations of the entire moist diet. Sulfadiazine was too insoluble to dissolve completely in water, so the drug was thoroughly mixed with the dry food and then added to the water.

Table I presents the average amounts of drug ingested on the preferred diets, namely, 2% sulfanilamide, 1.5% sulfapyridine, 2.0% sulfathiazole, and 0.4% sulfadiazine. The corresponding mean blood concentrations obtained at the time of inoculation which was 4 days after drug had been started are given also. Since equipment for micro determination of drug concentration was not available, it was necessary to kill the animals used for each blood determination. Blood was drawn for these determinations at the same time of day that inoculations were always made, 3-5 P.M. The mean drug concentration in the blood and the corresponding drug intake per day $\pm$ standard error have been calculated from determinations made on groups of 15-20 animals. The mice (15-22 g) were kept in individual cages and the food cups were weighed daily.[§]

The animals were inoculated intramuscularly in the inner aspect of the thigh 4 days after drug feeding had begun, and the drug was continued 7 and 14 days after inoculation.

The strain of *Cl. welchii* used was one which had been isolated from a patient who had a fatal postpartum infection. Inoculation in mice regularly produced a generalized

infection accompanied by necrosis of the thigh muscle at the site of inoculation. An almost uniform virulence for mice was obtained over a period of several months by the following standardization of technic: The original isolation culture was kept in the refrigerator in Rosenow's brain broth. Each culture used for inoculation was prepared by starting with a transfer from this original broth. Transfers were then made daily for 7 days. The last transfer was made into a tube containing brain and 9 cc of broth. The 18-hr growth from this tube was diluted 1:4 with physiological saline and 0.25 ml (0.0625 ml undiluted culture) was inoculated into the thigh muscle. An attempt was made to use an inoculum sufficient to kill approximately 80% of the untreated animals. After the dose had been determined it was further controlled by the inoculation of 10 control animals with each series of drug-fed animals.

The results reported here are the combination of experiments which yielded very similar results on repeated trials. As can be seen, sulfanilamide was of little or no value, sulfapyridine was somewhat better, but results were not consistent, sulfathiazole gave good results, but sulfadiazine was the best.

These results are considerably better than those reported in a similar study made by Bliss, Long and Smith.¹ It is quite possible

TABLE II.
Comparison of Sulfonamides in *Cl. Welchii* Infection.

Diet	No. of animals	% survivals
Untreated controls	75	15.7
2% sulfanilamide	44	27.2
1.5% sulfapyridine	48	46.4
2% sulfathiazole	54	79.6
0.4% sulfadiazine	46	93.4

[§] We are indebted to Dr. R. N. Bieter for full information on the preparation of cages and food cups.

that this difference is due to the fact that we produced drug concentrations of 11-19 mg % in the blood of our animals whereas they produced concentrations of only 5-7 mg %.

Although the animals were given drug before inoculation, the experiments cannot be considered entirely from the standpoint of prophylaxis, because all the animals showed a generalized infection 6-18 hr after inoculation, some of them developed hematuria, and all of them stopped eating or ate very little food for a day or two. If they survived this period, they usually remained well throughout the following 2-week observation period.

Even though the animals survived, the

tissue at the site of inoculation became necrotic and sloughed off. It made very little difference whether the drug was continued 7 or 14 days after inoculation, the lesions though walled off, did not heal. Vegetative organisms could be observed in direct smear and *Cl. welchii* were isolated in culture.

Conclusions. 1. Mice inoculated intramuscularly with *Cl. welchii* and treated orally with sulfonamides responded best to sulfadiazine (sulfadiazine 93.4%, sulfathiazole 79.6%, sulfapyridine 46.4%, and sulfanilamide 13.8% survival).

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Comparison of the Activity and Distribution of Iodine, in Reptilian and Mammalian Thyroids.

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The interesting observation that turtle or alligator thyroid had practically no effect on the basal metabolic rate of man was made by Swingle and Martin.¹ The iodine content of the thyroid preparations fed was very high, over 4 mg per g, and so, by mammalian standards, these glands should have been fully iodized and resting, with alveoli filled with dense colloid. Such thyroids have always been found to have strong physiological activity. In contrast to the action of these preparations on man, when they were given to rabbits, the metabolic rate was raised to about the same extent as when cattle thyroid was fed. Swingle and Martin attribute the different behavior of reptilian thyroid in man and the rabbit to the fact that the dose used for rabbits was relatively large.

Since this work was reported, methods for the accurate estimation of thyroxine in thyroid have been developed and these were applied to various samples of reptile thyroid, including those used by Swingle and Martin, to determine whether the distribution of

iodine differed from that found in mammalian thyroid. As a check on the validity of the analyses, the metamorphosing action of these preparations on tadpoles was studied and compared with the action of several dogs' thyroids.

Methods. Thyroxin was measured by Blau's² modification of the Leland and Foster³ method. In addition to the dried alligator and turtle thyroids used by Swingle and Martin, samples of which had been in this laboratory since Marine determined their influence on rabbit metabolism in 1926, we obtained thyroids of 4 Caribbean Sea turtles. These animals weighed between 70 and 110 kg and had been kept in a warm room on their backs for about 10 days before they were killed. The thyroids seemed somewhat edematous, an observation that is supported by the high water content found on drying the tissue in vacuum desiccators over BaO. A small piece of each specimen was fixed in formalin for microscopic examination.

² Blau, N. F., *J. Biol. Chem.*, 1935, **110**, 351.

³ Leland, J. P., and Foster, G. L., *J. Biol. Chem.*, 1932, **95**, 165.

¹ Swingle, W. W., and Martin, K. A., *J. Exp. Zool.*, 1926-7, **46**, 277.