and by Case,[4] has been considered but no final conclusion reached.

[3] Doniach, I., Grüneberg, H., and Pearson, J. E. G., *J. Path. and Bact.*, 1943, **55**, 23.

[4] Case, R. A. M., *Nature*, 1943, **152**, 599.

Whatever their nature, the association of these intra-erythrocytic inclusions with febrile anemia resistant to all known anti-anemic treatment is of considerable interest. Further studies are in progress, and will be reported in full elsewhere.

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Hemoglobin Regeneration in Dogs Receiving a Purified Ration Plus Succinylsulfathiazole.*

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Previous studies have shown that dogs grow quite well and regenerate hemoglobin very rapidly on a sucrose-casein-synthetic vitamin ration when the casein is not highly purified;[1] that blood regeneration on mineralized milk diets is not as rapid even when extra vitamins are added,[2] and that a deficiency of certain of the B-vitamins does not always respond rapidly to administration of the missing vitamin.[3] These variations raised the question as to whether the intestinal flora might not be modified sufficiently by different rations to bring about a decreased production of some unknown factors necessary for the dog.

An attempt was made, therefore, to produce a more consistent and more severe deficiency by adding succinylsulfathiazole to the basal diet and subjecting the dog to severe phlebotomy. Growth and hemoglobin regeneration were equally as good, if not better, in the dogs receiving the drug at levels up to 4% as in the control animals. Since these results are distinctly different from those obtained in rats, we are reporting them very briefly.

Experimental. The basal diet had the following composition: acid washed casein 19%, sucrose 66%, cottonseed oil 11%, salts IV 4%,[4] and was supplemented (on a per kilo body weight per day basis) with 3 drops of haliver oil, 100 γ thiamine and riboflavin, 60 γ pyridoxine, 2 mg nicotinic acid, 500 γ pantothenic acid, and 50 mg choline.†

Eight mongrel litter mates were used, 6 of which (327-332) were put on the experiment immediately after weaning and 2 (325, 326) at the age of 2½ months. They were divided into 4 groups carefully selected on the basis of size and previous weight gains. Dogs 325, 326, 328, and 329 received the basal ration with added succinylsulfathiazole; dogs 330 and 331 were fed the basal ration with succinylsulfathiazole and 2% liver extract (1/20 powder); dog 327, a negative control, received only the basal ration, while dog 332, a positive control, received 2% liver extract (1/20 powder) added to the basal ration. The succinylsulfathiazole was mixed in the ration at a level of 0.5% for 7 weeks, then raised to 1% for 5 weeks and finally increased to 2% for another 3 weeks. Three-cc blood samples

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[1] McKibbin, J. M., Schaefer, A. E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1942, **145**, 107.

[2] Potter, V. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1938, **126**, 155.

[3] McKibbin, J. M., Schaefer, A. E., Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **142**, 77.

[4] Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 657.

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TABLE I.
Hemoglobin Made per Kilo Body Weight per Week, on Different Levels of Succinylsulfathiazole (Sulfasuxidine).

Dog No.	Addition to basal diet	Levels of succinylsulfathiazole 0.5%	1%	2%	Avg for entire experiment
325	Sulfasuxidine		2.29	2.34	
326	"		2.63	3.18	
328	"	1.42	2.14	3.07	1.82
329	"	1.47	2.62	3.31	2.31
330	" + 2% liver extr.	1.92	2.49	3.56	2.28
331	" " " " "	1.59	2.79	1.93	1.81
327	None	1.95	2.41	4.00	2.31
332	2% liver extr.	1.71	1.99	2.68	2.02

were taken weekly from the radial vein for routine hemoglobin, hematocrit, and red blood cell determinations.[5]

Phlebotomy (from the jugular vein) was always heavy (30-40% of the total blood volume at each bleeding) and repeated every other day in order to reduce the hemoglobin level to 6% within one week. The amount of hemoglobin removed was determined as oxyhemoglobin by Evelyn's method; the hemoglobin made was calculated from the difference in total hemoglobin of the dog between one sampling and another, assuming 8% of the body weight as total circulating blood.

For 7 weeks, the dogs were allowed to grow without bleeding. During this period, dogs 325, 326, 328, 329 and 332 received 0.5% succinylsulfathiazole. The drug was then raised to 1% and all the animals bled to anemic levels. Five weeks later, when the hemoglobin level had returned to normal, succinylsulfathiazole was increased to 2% and, after an additional week, all the animals were again made anemic through bleeding. During the recovery period, the level of succinylsulfathiazole was raised to 4% for dogs 328 and 329. After 5 more weeks, these 2 dogs were bled for the third time to a low level of hemoglobin.

Results and Discussion. No differences were observed between the 4 groups of animals, either in rates of growth, hemoglobin regenerated, appetite or general state of health.

Table I shows the amount of hemoglobin made during the different stages of the experiment, calculated weekly per kilo body weight.

In all instances, the hemoglobin level of the dogs returned promptly to normal after cessation of bleeding (Fig. 1). No plateau in weight was observed. Appetite remained constant throughout. Calculation of the mean cell volume revealed no significant variations at any time of the experiment.

It is evident from these studies that succinylsulfathiazole given at levels up to 4% of the diet fails to affect adversely either growth or blood regeneration in the dog. Since levels as low as 0.5% of the drug are injurious to the rat,[6] one might conclude that the dog does not require fractions such as biotin and "folic acid" which have been shown to be essential to the rat through the use of sulfa drugs. However, it is more likely that succinylsulfathiazole does not modify the intestinal flora sufficiently to produce a deficiency of these additional factors in the dog. It is well known that sulfapyridine produces additional deficiencies in dogs suffering from a nicotinic acid deficiency.[7] Preliminary work indicates that the feeding of sulfathiazole to nicotinic acid-deficient dogs produces similar conditions, but that succinylsulfathiazole has no adverse effect.‡

Although the casein used in these studies was the same as that used in our studies with

[5] Spector, H., Maass, A. R., Michaud, L., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1943, **150**, 75.

[6] Ransone, B., and Elvehjem, C. A., *J. Biol. Chem.*, 1943, **151**, 109.

[7] Schaefer, A. E., McKibbin, J. M., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 679.

‡ Krehl, W. A., and Elvehjem, C. A., unpublished data.

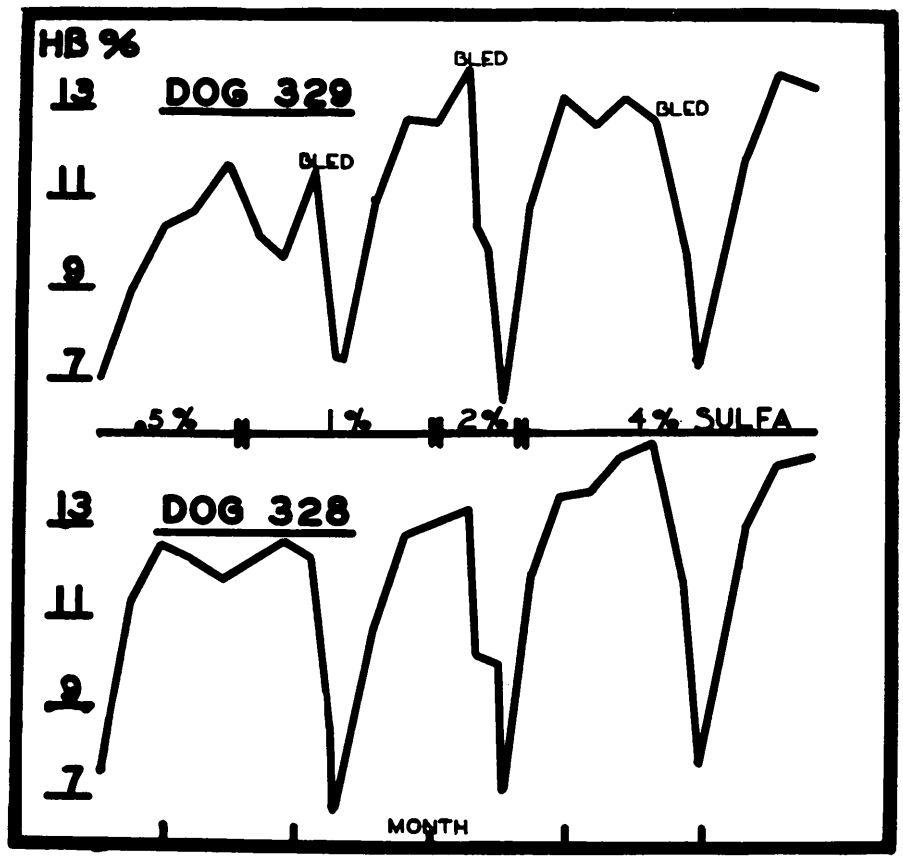

FIG. 1.
Typical hemoglobin picture of 2 dogs receiving varying levels of succinylsulfathiazole in their ration.

rats, it is possible that a deficiency would have been produced if the casein had been more highly purified. Lambooy and Nasset[8] have recently produced definite deficiency symptoms in young dogs by feeding a synthetic diet containing purified casein and sucrose but no sulfa drug. However, their ration contained added inositol and *p*-aminobenzoic acid which may have altered the intestinal flora. Smith[9] has also reported an anemia in dogs on a synthetic ration but again inositol and *p*-aminobenzoic acid were added. Thus, intestinal synthesis may be as important in dogs as in rats but the flora is not altered as readily by succinylsulfathiazole.

Summary. Succinylsulfathiazole was fed to dogs at levels varying from 0.5% to 4% of the ration in an attempt to modify the intestinal flora and cause a deficiency of factors possibly synthesized in the digestive tract.

No differences were observed between the animals receiving the drug and those serving as controls. Hemoglobin per 100 g of blood, total hemoglobin in the dogs, red blood cells, mean cell volume, food consumption, growth increase and general state of health were taken into consideration.

8 Lambooy, J. P., and Nasset, E. S., *J. Nutrition*, 1943, **26**, 293.

9 Smith, Susan Gower, *Federation Proc.*, 1944, **3**, 96.