7946 C

A Synthetic Vitamin A-Free Milk Suitable for Vitamin A Studies in Very Young Puppies.

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Busson and Simonnet[1] have shown that newly-born puppies have a relatively low reserve of vitamin A at birth and that at the time of weaning the reserve is usually only moderately higher. These results are in agreement with my own observations (to be published). Older dogs have a still higher reserve of vitamin A in the liver. In experiments (to be published) to determine the dog's vitamin A requirements, it appeared desirable, therefore, to use newly weaned puppies in order to shorten the necessary period of depletion and especially in order to be able to make some comparison with the vitamin A requirement of growing albino rats.

In the early attempts to feed to newly weaned puppies the usual dry or semi-solid vitamin A-free diet, a rather large number of animals was lost. In the experiments here reported, a synthetic bitch's milk was used, the composition of which was like that given for the natural product by Heineman:[2]

		Composition of synthetic milk	Parts
Specific gravity	1.035	Vitamin A-free soluble casein	110
Water	75.44	Crisco	95
Total proteins	11.17	Sucrose	30
Fat	9.57	O. M. salt mixture	7.5
Sugar	3.09	Linoleic acid	1.0
Ash	0.73	Tap water to make	1000
Total solids	24.56		

The milk was produced by the use of "crisco", linoleic acid, sucrose, Osborn-Mendel salt mixture and soluble vitamin A-free casein. Tap water was added in a quantity to give the same high total solid content of bitch's milk. This mixture was homogenized at 3000 lbs. pressure, placed in hermetically sealed cans and sterilized in the same manner as evaporated milk (240°F. for 15 minutes, allowing 20 minutes for coming up time and using a sterilizer with revolving reel to keep the cans in constant motion during sterilization). A supplement of dried brewer's yeast as a source of the B complex and of irradiated baker's yeast as a source of vitamin D was added before feeding.

The synthetic vitamin A-free milk described above has been used successfully in experiments on 20 puppies. No difficulty was

[1] Busson, A., and Simonnet, H., *C. R. Soc. Biol.*, 1932, **109**, 1253.

[2] Heineman, P. G., Milk, W. B. Saunders Co., 1921.

encountered in depleting the puppies. Xerophthalmia and other characteristic signs of vitamin A deficiency were obtained. Typical weight curves are given. The weight curves (Fig. 1) and general appearance of the animals (Fig. 2) indicate that this synthetic vitamin A-free diet is satisfactory for the purpose.

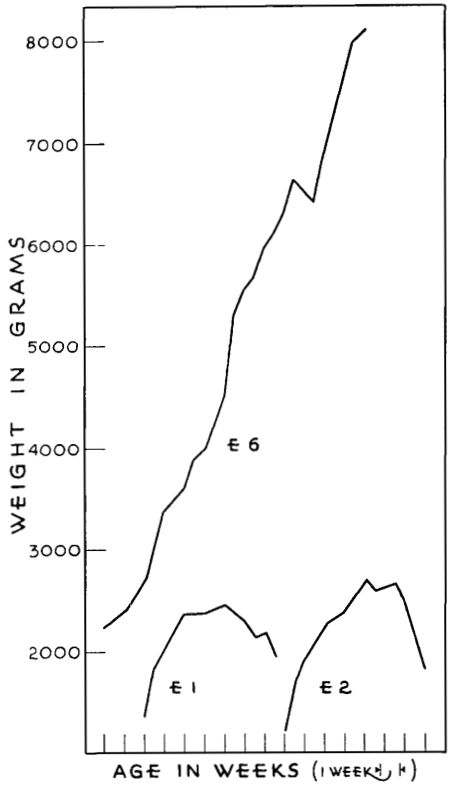


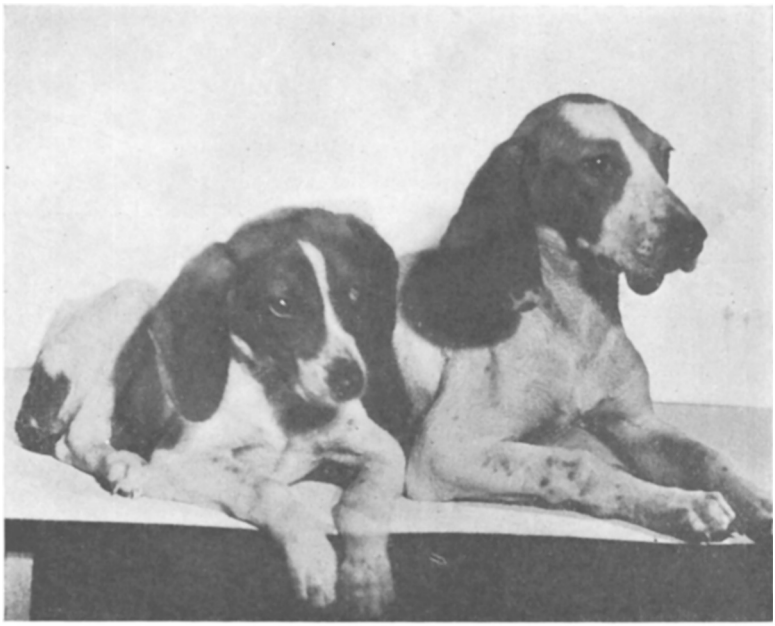
FIG. 1.

Growth curves of 3 puppies from one litter fed the vitamin A-free synthetic milk from time of weaning. E-1 and E-2 received synthetic milk only. E-6 received synthetic milk plus a daily supplement of 6300 new U.S.P.X units of vitamin A in the form of 0.3% solution of Carotene in cottonseed oil.

Two puppies have been given this milk supplemented with dried brewer's yeast, irradiated yeast and carotene in cottonseed oil for as long as 8 months.

The growth curves and general condition of those animals indicated that the milk so modified was complete for the young puppy for the first 2 or 3 months, during which time the diet consisted exclusively of this milk.

As the animals became older, additional carbohydrate was given



A B

FIG. 2.

Litter mates fed on vitamin A-free synthetic milk. "A" received synthetic milk only. "B" received synthetic milk plus 6300 new U.S.P.X units of vitamin A in the form of 0.3% solution of Carotene in cottonseed oil.

(a) (b)

FIG. 3. This is Puppy A of Fig. 2.

(a) Xerophthalmia of left eye after 10 weeks on the vitamin A-free synthetic milk.

(b) The same puppy after receiving a supplement of 2800 new U.S.P.X units of vitamin A in the form of 0.3% solution of Carotene in cottonseed oil for 13 days.

in the form of boiled polished rice to which was added an additional amount of dried brewer's yeast. Several other puppies were fed this completed milk for shorter periods with satisfactory results.

Summary. A synthetic milk has been produced that has been found satisfactory for vitamin A studies on young puppies. This milk could also serve for studies on vitamins B, G, and D.

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Preliminary Observations on the Frei Test in Lymphogranuloma Inguinale.

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Since the Frei test is considered to be allergic in nature a passive transfer in the sense of Prausnitz and Küstner was attempted. Five non-tuberculous individuals who had reacted negatively to Frei antigens and one Rhesus monkey were intradermally inoculated in different skin areas of the back with 0.1 cc. Frei antigen, 0.1 cc. inactivated normal human serum, 0.1 cc. sera of typical cases of Lymphogranuloma inguinale and 0.1 cc. mixtures of normal human serum and Frei antigens. Twenty-four hours later 0.1 cc. of a potent Frei antigen was injected into the same areas but with negative results. This was repeated in 3 patients at the end of 1 and of 2 months, again with negative findings.

The Frei test as now used is often difficult to evaluate. It is evident from the reactions that the antigen far exceeds the necessary dose for a more specific and less potent reaction and that the antigens may also contain non-specific factors.

Samples of Lymphogranuloma inguinale pus were dried *in vacuo* above anhydrous $CaCl_2$, ground to a fine powder and weighed. It was found that 0.1 gm. suspended in 10 cc. of physiologic salt solution (1:100) and heated at 60°C. gives an excellent antigenic emulsion. A positive reaction was secured in one case in a dilution as high as 1:20,000.

However, the grading off usually occurred between 1:5,000-1:10,000. Therefore, the test can be performed with much more dilute antigens than are at present in use. High speed centrifugations of the antigens yielded non-reactive supernatant liquids. Berkefeld filtrates also gave negative results.