Function of Egg White in Embryonic Development. (17474)

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In an attempt to decide whether or not the yolk or the egg white is the carrier of a growth factor transmitted through the egg from the hen's feed to the chick, the experimental technic described here was devised. In the past it has been assumed that egg white has a purely nutritional function in the growth and development of the chick embryo, but no experimental evidence exists to support this supposition.

The yolk also furnishes food, but in addition the yolk-sac serves as an organ for the digestion and assimilation of food by the chick embryo. This function of the yolk-sac indicates a structural relationship between the embryo and the yolk.

In this experiment fertile hen's eggs from crosses of Rhode Island Reds and White Leghorns were used. A circular window approximately ¼ inch in diameter was bored in the small end of each egg and the egg-white was removed into a glass ground-joint wash bottle by suction. To combat bacterial infection 0.1 cc of an aqueous solution containing 3000 I.U. of penicillin and 3.3 mg of streptomycin was injected into each of the eggs so treated. The egg white from another fertile egg was then transferred by air pressure from the wash bottle into the egg from which the egg white had previously been removed. When one egg white was not sufficient to completely refill the shell, that from another fertile egg was added. Thus, at times egg white from more than 1 egg was used for replacement in a single egg.

After the egg was completely filled with egg white, the window in the shell was closed with a strip of scotch tape and the egg placed into an incubator for 3 weeks. At intervals of 7 days, the condition and development of the chick embryo was observed by candling the eggs.

In the first successful series, 12 such eggs were prepared; of these 3 chicks hatched which were normal in every respect. Of the rest of the embryos, two lived 2 days, one 4, two 6, one 7, one 13, one 15 and one 21 days.

These are probably the first live chicks produced after such drastic treatment. This experiment demonstrates that the chief function of the white of the hen egg is mainly to furnish food for the developing embryo.

It has been previously held that the two chalazae serve to orient the embryo in a proper position for hatching by keeping the yolk more or less fixed. In these experiments, however, the two chalazae were removed with the egg white and thus the position of the developing embryo depended upon gravity. Further work is in progress on the nutrition of the chick embryo employing this technic.

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Distribution of Vitamin B_{12} in Natural Materials.* (17475)

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The increasing use of vitamin B_{12} in the treatment of human macrocytic anemias and in certain natural rations for farm animals makes the knowledge of the distribution of this vitamin in biological materials of real importance. A rat assay for vitamin B_{12}, based on the fact that thyroid active materials increase the requirements for this factor, has been used with considerable success in our laboratory.[1,2] With the availability of pure vita-

TABLE I.
Vitamin B_{12} Content of Natural Materials.

Daily supplement	Avg growth 2 week assay period, g	Min. vit. B_{12} content fresh basis, μg/100 g	Min. vit. B_{12} content dry basis, μg/100g
None	35 ± 5		
0.1 μg vit. B_{12}, inj.	66		
0.1 μg vit. B_{12}, orally	65		
1 mg thymidine, inj.	36		*
2 mg thymidine, orally	36		*
250 μg thymine, uracil, adenine, guanine, and 500 μg rutin and hesperidin	35		*
10 ml tomato juice, fresh	34	*	
6% dried streptomycin "slop"	95		>22
6% condensed fish solubles	96	20	>40
5% herring stickwater	55	7	14
5% desiccated hog mucosa	43		trace
2% desiccated swine adrenals	57		15
1.25% desiccated sheep rumen contents	76		50
2.5% desiccated sheep rumen contents	93		50
10% crude casein	53		3
10% purified casein (vitamin test)	15		*
10 ml raw milk (cow)	41	trace	
20 ml raw skimmed milk (cow)	42	trace	
25 ml raw milk (goat)	28	*	
15% whole milk powder (Klim)	61		2.5
Rennet ppt. casein 25 g milk†	50	0.12	1
15% malted milk	54		2.2
2.5 g cheese cheddar	56	1.4	2.3
4 g egg yolk	64	1.4	2.8
1 g beef liver (fresh)	82	15	>50
1 g beef kidney (cooked)	90	20	>50
.125 g viobin liver powder, 37°‡	80		100
.125 g viobin liver powder, 70°C‡	78		97
5 g beef round, sample 1 (cooked)	74	2	3.6
5 g beef round, sample 2 (cooked)	83	3	5.5
2.5 g beef round, sample 2 (cooked)	68	3	5.5
5 g beef tongue (cooked)	91	3	5.5
.75 g chicken liver (fresh)	70	11	35
5 g pork shoulder, sample 1	73	2	3.6
5 g pork shoulder, sample 2	67	1.2	2.2
5 g pork hocks	51	0.5	0.9
5 g pork ham	68	1.2	2.2
5 g mutton	82	3	5.5
5 g veal	76	2	3.6
5 g horsemeat (canned)	84	3.4	7.5

* No measurable quantity.
† Calculated on fresh milk basis.
‡ Defatted and desiccated.

min B_{12} it is possible to supply standard amounts to rats and thereby calculate the actual amount of vitamin B_{12} present in the samples tested.

We wish to present in this paper a preliminary report of the distribution of vitamin B_{12} in a variety of biological materials.

Experimental and Results. The assay procedure has been described in detail in a previous paper.[1] Male weanling rats are placed on a corn-soybean-basal ration containing 0.1% iodinated casein† for a 2 week depletion

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1 Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.*, 1949, **177**, 129.

2 Register, U. D., Lewis, U. J., Thompson, H. T., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 167.

† Supplied by Dr. W. R. Graham, Cerophyl Laboratories, Inc., Kansas City, Mo.

period. At the end of this time supplements are administered for another 2-week period during which time the growth response is followed. The vitamin B_{12} content of the sample is calculated through the use of a standard growth curve plotted from data obtained with graded levels of crystalline vitamin B_{12}.[2]

Five rats were used for each assay and an average of the growth response for these rats was taken. In most cases the material to be tested was added on a percentage basis to the basal ration and the vitamin B_{12} content was calculated from the total food consumed. In other cases, due to the nature of the material, the supplement was given in separate food containers and the amount consumed was measured directly. The results are summarized in Table I.

Average growth responses on the basal ration during the 2 week assay period have been found for a large number of animals to be 35 g ($\pm$ 5 g). The daily administration of 0.1 μg of vitamin B_{12} either orally or by injection, increased the growth response to 65-66 g. Since Hall, *et al.*[3] showed that incubation of vitamin B_{12} with gastric juice increased the hematopoietic activity in pernicious anemia when administered orally, a similar treatment was tried for the rat. No effect on the activity of vitamin B_{12} was noted after incubation with human gastric juice.

Thymidine has been found to be capable of replacing vitamin B_{12} in the nutrition of *Lactobacillus lactis*.[4,5] Results presented in Table I show that this compound has no activity in the rat assay. Fresh tomato juice, which has been found to contain the TJ factor required by *Lactobacillus lactis* Dorner,[6] was likewise found to be inactive. Furthermore, a combination of thymine, uracil, adenine, guanine, rutin and hesperidin produced no growth response. It appears, therefore, that the rat assay is rather specific for vitamin B_{12}.

Two samples of yeast autolysates, U2 and Z7, supplied by Dr. C. N. Frey, Standard Brands, Inc., were found to be completely inactive. A sample of "slops" from streptomycin production was found to be very high in vitamin B_{12}. The regular commercial condensed fish solubles showed high activity while a sample of herring stickwater was not as active.

Sheep rumen contents were very active, which suggests a synthesis of this vitamin within the rumen. Milk, cheese, meats and egg yolk were found to be relatively good sources of the vitamin. Dried soybean sprouts, barley, fresh potatoes, beans, cabbage, green peas, alfalfa leaf meal, and fresh grass contained no significant amount.

The vitamin B_{12} content of beef kidney was found to be as high as that of beef liver. This is in agreement with previous results obtained on the vitamin B_{12} content of liver and kidneys from rats fed different rations.[7] The higher values obtained with the two liver powder preparations of the VioBin Corporation may be attributed to the fact that they were defatted and also that the fresh liver was fed at a near maximum level. However, more samples must be tested before definite conclusions can be made. It can be seen from the results that drying the liver at 70° did not noticeably alter the vitamin B_{12} content. The variations in pork samples obtained in this work and in previous work[2] are undoubtedly due to the fact that swine are monogastric animals and therefore do not benefit from the nutrients that may be produced by microorganisms in the rumen of cattle. The high level found in horse meat indicates that the synthesis of vitamin B_{12} in the cecum of the horse may be as efficient as in the rumen of cows and sheep.

Summary. The vitamin B_{12} content of a number of natural materials as determined by the rat assay is presented. Fish solubles, streptomycin "slops", sheep rumen contents and glandular meats are excellent sources of vitamin B_{12}. Muscle tissue, eggs and milk

[3] Hall, B. E., Morgan, E. H., and Campbell, D. C., *Proc. Staff Meet., Mayo Clin.*, 1949, **24**, 99.

[4] Shive, W., Ravel, J. M., and Eakin, R. E., *J. Am. Chem. Soc.*, 1948, **70**, 2614.

[5] Wright, L. D., Skeggs, H. R., and Huff, J. W., *J. Biol. Chem.*, 1948, **175**, 475.

[6] Short, M. S., *J. Bact.*, 1947, **53**, 669.

[7] Lewis, U. J., Register, U. D., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 509.

products contain lesser amounts, whereas plant materials show no measurable activity.

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Observations on Diaphragm and Stomach of the Dog Following Phrenicotomy.* (17476)

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In a recent communication we have pointed out some of the relationships between the left diaphragm and gastro-intestinal mechanisms in the dog.[1] The significant feature of these observations was a decrease in gastric emptying time in the presence of a relatively dilated stomach after left sided transthoracic phrenicotomy. The dogs were kept for prolonged periods in order to observe whether the denervated diaphragm would resume its respiratory function. This was done in view of Noack's work, who reported that regular rhythmic respiratory contractions of the diaphragm reappeared in the human from one and a half to two years after phrenic exeresis in the neck.[2] Noack's statement has been accepted by a number of authors. The mechanism of recovery of diaphragmatic function was postulated to be through the twelfth thoracic intercostal nerves and through sympathetic innervation. If the innervation of the diaphragm and recovery of its function following interruption of the phrenic nerve in man and dog are comparable, experiments on the latter offer the advantage of better control of most experimental conditions and ensure a complete section of the phrenic nerve, which is not always possible in the usual operations in the neck of man for phrenic exeresis. Long term observations comparable to those done by Noack on the human were done on the dog. The animals were observed fluoroscopically, in order to determine if and when there would occur reactivation of the diaphragm after complete left transthoracic phrenic section, the right diaphragm serving as normal control. Sauerbruch[3] described a gastrocardiac symptom complex following left side phrenic exeresis, which was later called Roemheld's syndrome. The symptoms were bradycardia, premature systoles, anxiety, epigastric distress, lack of appetite, pallor, nausea, vomiting and eructations.

Experimental. Sixteen dogs were operated upon transthoracically under aseptic conditions, using pentobarbital sodium anesthesia. In 13 animals a section of the left phrenic nerve from the hilum of the lung to the diaphragm was removed. Two dogs had simple phrenic section at the level of the diaphragm, and one had the nerve cut at the hilum of the lung. The cut ends of the nerve were doubled up and tied with non-absorbable suture. The dogs appeared normal after a few days of postoperative care. All dogs were fluoroscoped at weekly intervals for the first 12 to 14 weeks after operation and then at monthly intervals, to watch for possible reactivation of the diaphragm and for the possible development of Roemheld's syn-

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1 Jefferson, Nelson C., and Necheles, H., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 166.

2 Noack, R., *Beitr. Z. Klin d. Tuberk.*, 1933, **82**, 397.

3 Quoted by Roemheld, L., *Münch. Med. Wchnschr.*, 1928, **75**, 1872.