chick embryo tissue culture medium. It was surprising, however, to find that when dead embryos were incubated at 35°C for a sufficient period the virus titer became much higher than is found in liquid tissue culture.[6] The evidence obtained does not suggest that the virus is capable of multiplying in the absence of living cells. It has been demonstrated clearly[1,2] that in embryos killed by chilling at 4°C or by prolonged storage at room temperature, living cells are present. Presumably these cells carry on their usual metabolic functions when held at 35°C. It seems probable that the long period of incubation necessary for the development of maximal virus titers may be related to and dependent upon the multiplication of such living cells in both the dead embryo and its membranes. In this connection it will be recalled that when embryos were frozen at –30°C they were thereafter incapable of supporting viral multiplication.

It is apparent that these results may be of practical usefulness, especially in field work where attempts are made to recover viruses from infected persons or animals. In the case of influenza virus, at least, it seems evident that it is not necessary to observe special precautions with chick embryos. When adequate laboratory facilities are lacking, dead embryos which have been stored for considerable periods may prove satisfactory for the recovery of the infectious agent.

Summary. Chick embryos killed by prolonged storage at room temperature or 4°C are capable of supporting the multiplication of influenza virus upon prolonged incubation at 35°C.

6 Weller, T. H., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 124.

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Relation of Vitamin B_{12} to Liver Basophilia.*

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Vitamin B_{12} has been reported to stimulate the growth of chicks on a vegetable protein diet and of certain lactobacilli.[1,2] When it was later found that the nucleoside thymidine can replace vitamin B_{12} in the medium of these microorganisms, it was postulated that the vitamin acts as an enzyme in the synthesis of nucleosides and thus of nucleotides and nucleic acids.[3,4] It was decided to test this theory by studying the formation of nucleic acids in the rat as influenced by vitamin B_{12}. Since it is generally accepted by histochemists that the basophilia reaction determines ribonucleoprotein,[5] the effect of vitamin B_{12} on the basophilia of rat livers was studied in this experiment.

Experimental. Weanling white rats whose mothers had been fed a diet in which the sole source of protein was soybean oil meal were used in this investigation. When the young were 21 days old they were removed from the mothers and divided into 3 groups. Groups 1 and 2 received a diet of the purified type in which soybean oil meal was the sole source

* Journal Series paper of the New Jersey Agricultural Experiment Station, Rutgers University, the State University of New Jersey, Department of Agricultural Biochemistry.

1 Ott, W. H., Rickes, E. L., and Wood, T. R., *J. B. C.*, 1948, **174**, 1047.

2 Shorb, Mary L., *Science*, 1948, **107**, 397.

3 Wright, L. D., Skeggs, Helen R., and Huff, J. W., *J. B. C.*, 1948, **175**, 475.

4 Shive, W., Ravel, Joanne M., and Eakin, B. E., *J. Am. Chem. Soc.*, 1948, **70**, 2614.

5 Demsey, E. W., and Wislocki, G. B., *Phys. Rev.*, 1948, **26**, 1.

TABLE I.
Relation Between Diet and Basophilia.

Group	Rat No.	Dietary supplement	Gain, g per day	Basophilia, grade
1	1	None, negative control	0.8	0
	2	" " "	1.2	0
	4	" " "	1.0	+
	5	" " "	2.5	0
	22	" " "	0.9	+
	28	" " "	1.3	0
	29	" " "	0.6	0
	36	" " "	1.0	0
2	3	B_{12}	4.2	+++
	6	"	2.4	+++
	24	"	4.3	+++
	39	"	4.4	++
3	27	Liver	5.1	++++
	34	"	4.0	++++

of protein. Vitamin B_{12}† was included in the diet of Group 2 at a level of 440 μg per kilo. Group 1, the diet of which was not supplemented, served as the negative control. For Group 3, the diet was modified by the introduction of 4.2 g of dried liver containing 2.5% protein per 100 g of diet at the expense of the soybean oil meal. All diets contained 22% protein and included vitamins and salt mixture in optimal amounts. The pups were allowed to feed and drink *ad libitum* and were sacrificed at the end of the 21-day experimental period.

Segments of liver were removed immediately after the animals were sacrificed, fixed in Carnoy's fluid, dehydrated through alcohol, and sectioned at 8 μ. The sections were stained with eosin and methylene blue.

Results. An arbitrary scale was decided upon to evaluate the extent of the basophilic staining. Livers which showed no cytoplasmic basophilia were graded 0, and those which exhibited minimal basophilia were graded +. Moderate concentrations were graded ++; sections with more intensive staining were graded +++; and those sections whose cytoplasm was replete with basophilic staining were graded ++++.

Table I shows that rats which received vitamin B_{12} or dried liver grew at a markedly greater rate than those which received no supplement. Furthermore, the animals which gained slowly were characterized by little or no hepatic basophilia, whereas those which gained rapidly showed extensive cytoplasmic basophilia in the liver cells.

The review by Demsey and Wislocki[5] points out that basophilia, shown to be ribonucleoprotein, is intimately related to protein synthesis. It would thus seem that when animals are deprived of vitamin B_{12} the formation of nucleoprotein (through the nucleoside) is limited, and consequently protein synthesis in the body would be retarded.

Summary. The growth of weanling white rats was stimulated by inclusion of vitamin B_{12} or of whole dried liver in the diet. Rats which received no B_{12} or liver grew poorly and showed little or no liver basophilia, whereas those which received B_{12} or liver grew well and showed considerable cytoplasmic basophilia in their liver cells.

† Vitamin B_{12} concentrate was kindly supplied by Merck & Company, Rahway, N. J.