phase. (3) The reticulo-endothelial cells of such perfused livers retain their ability to remove particulate matter from the perfusate stream. (4) Bromsulfalein is extracted from the perfusate by the isolated liver, and is re-excreted into the bile in high concentration and in several separable components, analogous to those found in the intact animal. (5) Data have been presented to permit a comparison of the conditions of operation and the performance of the isolated rat liver, and the liver *in situ* in the intact animal. (6) Details of the method of preparation, the perfusion pump, and the perfusion media have been presented, together with specific data concerning the course of typical experiments. (7) The range of applicability of the preparation in its present state of development and directions of further exploration have been discussed.

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Nucleic Acid Changes in Rat Nerve Tissue After Parenteral Administration of Vitamin B_{12}. (19013)

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The beneficial effects of vit. B_{12} in treating nutritional anemias(1,2) and neurological disturbances(3,4) have been demonstrated. The biochemical mechanisms and the cytological localization of the activity of this vitamin as yet are not fully known. Facilitation of the formation of ribonucleic acid in liver cells protected by large doses of vit. B_{12} against depletion by poisoning with carbon tetrachloride has been reported by Koch-Weser and Popper(5). On the assumption that the intensity of the basophilic reaction indicates the quantity of nucleoprotein present, Stern, Taylor, and Russell(6) studied weanling rat livers stained with methylene blue. These investigators found that there was almost no basophilia in rats on a diet containing soybean oil meal as the only protein source. The addition of vit. B_{12} to this diet produced a decided liver basophilia. Liver extract gave a slightly greater response. In 1950, after our work had begun, Seigel and Worley(7), using basophilia as an index of the presence of nucleic acids, confirmed the results of Stern *et al.* A nucleic acid abnormality in bone marrow cells due to pernicious anemia also has been corrected by local injection of vit. B_{12}(8). The present investigation has been undertaken to determine whether parenteral administration of B_{12} produces definite cytochemical changes in the nucleic acids in nerve tissue in rats. Several stains were used to visualize any possible changes in basophilia, total nucleic acids, pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA).

Materials and methods. Two experiments were carried out. Table I contains a summary of the dietary groups and the quantities of vit. B_{12}* administered. Food consumption records were kept daily and the animals weighed every other day. The basal diet was

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* The vit. B_{12} used in this study was furnished through the courtesy of Mr. A. W. Veazey of Merck & Co.

TABLE I. Summary of Experimental Groups and Quantities of Vit. B_{12} Administered Subcutaneously.

Exp.	Dietary groups	No. of rats	Parenteral B_{12}
1*	Chow diet†	4	Control
	" "	4	.25 μg B_{12} dieb. alt. 20 days
	Basal " ‡	4	Control
	" "	4	.25 μg B_{12} dieb. alt. 20 days
2	Chow diet + .5% sulfa	4	Control
	" " " 2% "	4	"
	Basal " " " "	3	"
	" " " " "	1	2 μg B_{12} dieb. alt. 4 days;
	" " " .5% "	2	4 μg B_{12} dieb. alt. 6 days; last dose 10th day 7 μg B_{12}

* The diets of all the rats in Exp. 1 were supplemented with .5% sulfa on 13th day of exp.
† Purina laboratory chow.
‡ Alcohol extracted casein 18, sucrose 68, corn oil 1, cellu flour 2, salt mixtures 3A 2 and 3B 2(9), vit. B complex mixture 5(9), and fat soluble vitamin mixture 2%(9).

deficient in vit. B_{12}, folic acid and para-aminobenzoic acid (PABA). By the 13th day of the first experiment the weanling rats on chow diet plus B_{12} were gaining weight more rapidly than those on unsupplemented chow diet. Those on the basal diet weighed slightly more than those on the basal plus B_{12}. In order to prevent possible synthesis of B_{12} due to intestinal bacteria, the diets of all the animals were supplemented with 0.5% sulfaguanidine (sulfa), to alter the intestinal flora. Growth rates were lessened for all groups. The discrepancy between the rats on basal diet and those on basal plus B_{12} was increased. All of these animals were sacrificed on the 20th day of the experiment. The 9-week-old rats† used in experiment 2 had been on a diet supplemented with either 0.5% sulfa or 2.0% sulfa, but deficient in vit. B_{12}, folic acid, PABA and vit. K for 6 weeks before we received them. At that time a group of 4 animals on chow diet plus 0.5% sulfa and another group of 4 on chow plus 2.0% sulfa were sacrificed. The remaining rats were divided into 2 groups and placed on the basal diet deficient in vit. B_{12}, PABA and folic acid supplemented with sulfa. The starting dosage of vit. B_{12} for the one animal on 2.0% sulfa and the 2 on 0.5% sulfa was 2 μg every other day for 4 days. Since these already stunted rats showed no growth response they were given 4 μg of B_{12} every other day for a total of 3 injections. On the 10th day they were each given 7 μg of B_{12} and sacrificed about 5 hours later along with the 3 rats which had been retained on the deficient diet plus 2.0% sulfa without parenteral B_{12}. Rats from both experiments were killed by a severe blow on the head. Tissues were placed immediately in Zenker's fixative and subsequently paraffin embedded. 10 μ sections of spinal cord, autonomic ganglia (cervical trunk) and liver from all animals have been examined.

Four stains were used on all tissues: 1. Hematoxylin and eosin for general basophilia. 2. A modification of Turchini's 9-methyl-2, 6, 7-trihydroxy-3-fluorone for selective staining of both PNA and DNA(10). 3. Feulgen reaction(11). 4. Einarsson's gallocyanin-chrome alum. This combination of stains made possible an examination of the cellular basophilia as well as of the PNA and DNA collectively and separately. Digestion of liver, spinal cord and autonomic ganglion sections with ribonuclease (Worthington) provided for a qualitative examination of the Turchini and Einarsson stains for PNA. Sections were placed in ribonuclease, 1.0 mg per ml of McIlvaine's buffer at pH 6.85 for 3 hours at 60°C. Control sections from the same block of tissue were placed in the buffered solution without the enzyme for the same time period.

† Medical students demonstrated avitaminoses with these rats as a part of their biochemistry course. Dr. E. A. Doisy, Jr., who supplied us with animals for the first experiment, was also kind enough to allow us the use of these rats.

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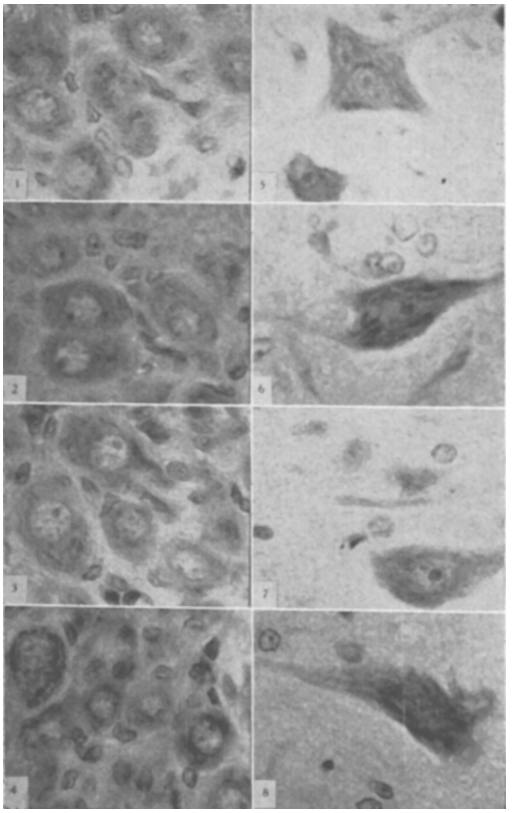


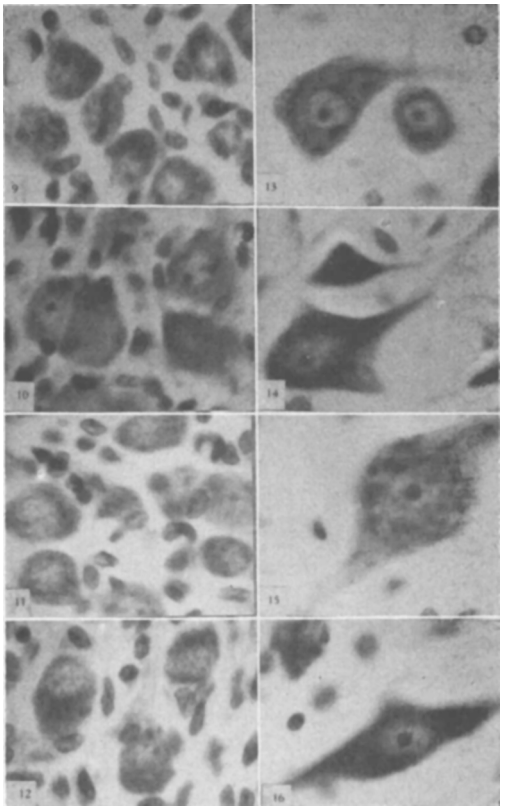
Turchini stain

FIG. 1. Autonomic ganglion, rat, chow diet.
FIG. 2. Autonomic ganglion, rat, chow diet + parenteral B_{12}.
FIG. 3. Autonomic ganglion, rat, basal diet.
FIG. 4. Autonomic ganglion, rat, basal diet + parenteral B_{12}.
FIG. 5. Anterior horn cells, rat, chow diet.
FIG. 6. Anterior horn cells, rat, chow diet + parenteral B_{12}.
FIG. 7. Anterior horn cells, rat, basal diet.
FIG. 8. Anterior horn cells, rat, basal diet + parenteral B_{12}.

The almost complete loss of cytoplasmic and nucleolar stain by the enzyme treated sections and the presence of the Turchini and Einarsson stains at the sites of PNA in the buffer treated sections is an indication of the qualitative reliability of these stains.

Results. There were no significant differences in the staining reactions when the tissues of one animal of a group were compared with the same tissues of other animals in the same group. There was a constant observable difference in the staining reaction when the same tissues from the various groups were compared, but no differences among animals within a group.

Our findings that staining with hematoxylin and eosin showed enhanced basophilia in the tissues studied in rats which had received B_{12} over those which had not, confirmed the results of previous workers(6,7). Because of the differentiation procedure involved in staining and because we are not convinced that basophilia, *per se,* is a positive indication of nucleic acid, these results were not considered significant. The Turchini stain produced consistent results. The demonstration of PNA



Einarsson stain

FIG. 9. Autonomic ganglion, rat, chow diet.
FIG. 10. Autonomic ganglion, rat, chow diet + parenteral B_{12}.
FIG. 11. Autonomic ganglion, rat, basal diet.
FIG. 12. Autonomic ganglion, rat, basal diet + parenteral B_{12}.
FIG. 13. Anterior horn cells, rat, chow diet.
FIG. 14. Anterior horn cells, rat, chow diet + parenteral B_{12}.
FIG. 15. Anterior horn cells, rat, basal diet.
FIG. 16. Anterior horn cells, rat, basal diet + parenteral B_{12}.

was clear cut. The tissues of the animals receiving B_{12} always showed more PNA than those of the controls (Fig. 1-8). Liver, anterior horn cells and autonomic ganglion cells all showed an increase in PNA. Definitive DNA changes in tissues stained by this method could not be observed although the localization, by color, was obvious. The presence of nuclear PNA was too distracting for an objective analysis of a cell's DNA content. Staining of these same tissues by the Einarsson method confirmed the results obtained through the Turchini procedure (Fig. 9-16). Since there is no color differentiation between PNA and DNA, attention was focused primarily upon the cytoplasm. Here one noted clear cut differences between preparations from B_{12} treated and those from control animals. Tissues of B_{12} treated rats always showed more PNA than the controls. The Feulgen stain indicated the presence of DNA. The same histological picture was seen, both quantitatively and qualitatively, for spinal cord and autonomic ganglia of all the animals. Preparations of liver showed a consistent, although slight, rise in DNA content in B_{12} treated rats. The results of the second experiment confirmed those of the first.

Summary. These preliminary results support the contention that vit. B_{12} is related to the genesis of ribonucleoproteins in rat tissues. Weanling and immature rats were placed in 2 dietary groups, chow and basal, the latter dietary group deficient in vit. B_{12}, folic acid and PABA. One-half of each group received parenteral B_{12} every other day. Liver, spinal cord and cervical sympathetic ganglia were examined histologically for changes in pentose and desoxypentose nucleic acids. These 3 tissues from animals which received vit. B_{12} showed more PNA than the controls. No consistent observable difference was seen in spinal cord and cervical sympathetic ganglia for the DNA as visualized by the Feulgen stain. There was a slight increase in the intensity of the Feulgen stain in hepatic cell nuclei from rats which had received vit. B_{12} over the controls.

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Studies on a Proteolytic Enzyme System of the Blood. IV. Activation of Profibrinolysin by Serum Fibrinolysokinase.* (19014)

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Recent studies in this laboratory concerning the activation of profibrinolysin (plasminogen) by fibrinolysokinases of bacterial(1,2) and tissue(3) origin have included observations which suggest that a kinase is also involved in the so-called "spontaneous" activation of serum profibrinolysin. Active fibrinolysin (plasmin) although not present in normal whole serum, has long been known to appear in certain serum fractions(4,5) and in whole serum after treatment with chloroform (6). Our working theory of the interactions of the various components of the fibrinolytic enzyme system of dog serum is:

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