much longer life span than the leucocytes, and it is probable that the polymorphonuclear leucocyte has a slightly longer life span than the lymphocyte. It is to be expected, therefore, that the percentage of new cells of each type in the circulating blood of a normal rat at a given time would decrease in the order mentioned above.

Thus, most of the lymphocytes are associated with autoradiographs. The polymorphonuclear leucocytes are associated in several cases with autoradiographs, despite the fact that these are the least numerous of the three cell types in the rat blood. The erythrocytes rarely produced an autoradiograph under our experimental conditions, despite their relatively large numbers in the circulating blood.

The grain concentration, *i.e.* number of silver grains per unit area, in the autoradiographs, which is a measure of the relative amounts of C^{14} incorporated in the cells, varies in each cell category. There are cells of each type which reveal no C^{14} incorporation detectable by this technique. Of those which yield autoradiographs, however, the concentration of silver grains is generally greatest in the case of the lymphocytes. Fig. 1, 2, represents approximately the average grain concentration in autoradiographs associated with lymphocytes. The maximum grain concentration in autoradiographs associated with polymorphonuclear leucocytes (Fig. 1, 3) is less than that found with most lymphocytes. The maximum grain concentration associated with erythrocytes (Fig. 1, 4) is less than that of most of the definite autoradiographs given by leucocytes.

The concentration of silver grains appears to be higher in the case of those cells containing relatively larger amounts of nuclear material. It seems reasonable to assume that the cells which show the presence of C^{14} have incorporated the labeled materials in their proteins. Since, according to Abrams, *et al.* (*1*), glycine is a specific precursor for purines of the nucleic acids of yeast, much of the C^{14} activity may reside in the purine moiety of the nucleoproteins. Inasmuch as the concentration of nucleoproteins is the highest in lymphocytes, this offers a possible explanation for the variation in grain concentration among the three cell types.

It is probable that glycine is incorporated into the hemoglobin of the red cell in the bone marrow and not in the circulating blood. This contention is supported by *in vitro* studies of London, *et al.* (*5*), which showed that the synthesis of heme from glycine does not occur to a detectable extent in normal human peripheral blood incubated with glycine labeled with N^{15}, and by the finding that rabbit bone marrow homogenates incorporate appreciable amounts of C^{14}-labeled alpha-carbon of glycine in hemin within 3 hrs of incubation (*3*). The present experiment strongly suggests, therefore, that the red blood cells associated with autoradiographs are cells which were recently formed and introduced into the circulating blood within the 25-hr period of the experiment.

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Histological Localization of Newly-formed Desoxyribonucleic Acid[1]

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The histological localization of newly-formed desoxyribonucleic acid was attempted by the use of the "radioactive autograph" technique in the tissues of animals treated with large amounts of radiophosphorus.

Female rats weighing from 50 to 70 gm were given a single subcutaneous injection of about 1 mc of P^{32} in a solution of H_3PO_4 containing 25 μg of phosphorus. The animals were sacrificed 2 or 24 hrs later. The tissues were fixed in neutral formalin, dehydrated in dioxane, embedded in paraffin, sectioned, and mounted on glass slides in the routine manner. After deparaffination the slides were treated for 1 hr at 40° C with a 0.05% solution of ribonuclease in citrate-phosphate buffer at pH 7, control slides being similarly taken through a buffer solution without ribonuclease. Half the slides were stained with hematoxylin-eosin, the others being left unstained. The slides were then coated with photographic emulsion according to the "coated autograph" method (*1*, *3*).

Most of the phosphorus compounds originally contained in the tissue sections were extracted during the preparation of the autographs. Thus, phospholipids were removed when the tissues were passed through several baths of dioxane and the slides through xylol and alcohol. Similarly, water-soluble phosphates, such as phosphate ions, hexose-phosphates, creatine-phosphate, were eliminated during either fixation, staining, or ribonuclease-buffer treatment. It was shown on control slides stained with pyronine that ribonuclease removed the cytoplasmic basophilia from pancreas and liver; therefore, ribonucleic acid was assumed to have been more or less completely extracted. It was concluded that desoxyribonucleic acid was the only phosphorus compound remaining in the sections in significant amounts. Autographs of such sections should reveal the localization of the desoxyribonucleic acid formed since the time of injection of P^{32}.

The newly-formed desoxyribonucleic acid was found to be abundant in lymphatic tissue. Thus, the reaction was pronounced in the cortex of the thymus (Fig. 1) and moderate in other lymphatic organs. The myelogenous tissue of the bone marrow and that normally found in

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the spleen and some lymph nodes of 50- to 70-gm rats reacted intensely.

A strong reaction was present in the ovary, where it was limited to the granulosa of some follicles (Fig. 2). The lack of reaction in other follicles (Fig. 2) may be due to their incipient atresia; or possibly such follicles may have reached full maturity.

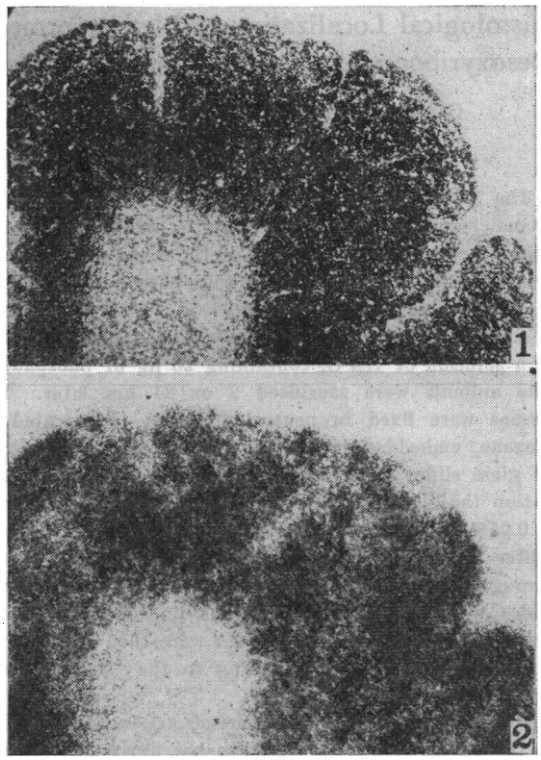

FIG. 1. (1) Thymic lobule of a rat sacrificed 24 hrs after P^{32} injection. A short exposure resulted in a slight autographic reaction which is hidden by the hematoxylin-eosin stain. Compare with (2), which is the same thymic lobule showing one of the following serial sections as an unstained autograph. The exposure was longer than in (1). The black reaction due to newly-formed desoxyribonucleic acid is intense in the cortex only. (×80.)

More or less intense reactions were present in many epithelial linings (intestine, stomach, esophagus, etc.). In contrast, most parenchymatous organs (pancreas, thyroid, etc.) and connective tissues showed no newly-formed desoxyribonucleic acid.

It should be emphasized that throughout the body the intensity of the reactions was roughly parallel to the mitotic counts, a fact which substantiates the theory (*2*) that phosphate ions enter the desoxyribonucleic molecule only at the time of mitosis.

Striking reactions were observed in the gastrointestinal tract. As early as 2 hrs after administration of P^{32} a definite reaction was noted in the crypts of Lieberkühn [Fig. 3 (5)], where mitoses are quite numerous. Twenty-four hours after injection an autographic reaction was found overlying the nuclei of the epithelial cells in the lower part of the villi [Fig. 3 (6)]. Apparently the radiophosphorus had been incorporated into the desoxyribonucleic acid synthesized by the dividing nuclei in the crypts; and the young nuclei, loaded with radioactive desoxyribonucleic acid, had ascended the sides of the villi. This observation confirmed a fact previously foreseen on theoretical grounds (*4*), that the cells of the epithelium covering the villi originate in the crypts.

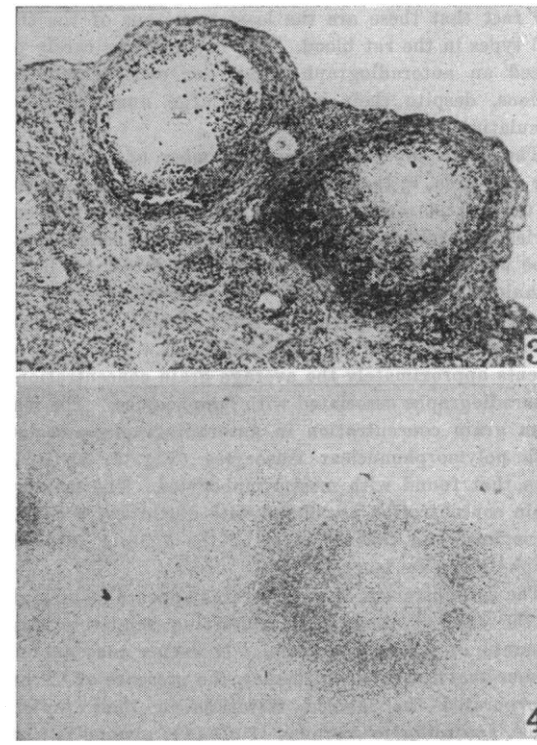

FIG. 2. (3) Ovary of a rat sacrificed 24 hrs after P^{32} injection. A short exposure resulted in a slight autographic reaction which is partly hidden by the hematoxylin-eosin stain. Compare with (4), the same region of the ovary, showing one of the following serial sections as an unstained autograph. The exposure was longer than in (3). The contrast between the right-hand side, reactive follicle and the left-hand side, nonreactive follicle is apparent. (×80.)

In both the fundic and pyloric regions of the stomach, a slight but definite reaction was noted at the bottom of the gastric pits. Mitoses were found in this region, presumably insuring a constant renewal of the epithelium covering the gastric mucosa.

Slight reactions were also noted in liver, kidney, and muscle, where practically no cell division occurs. There is a possibility that these reactions were not due to desoxyribonucleic acid but to traces of unextracted ribonucleic acid or other phosphorus compounds rendered insoluble by fixation. For example, adenosine triphosphate might be retained in tissue sections as adenosine diphosphate.

Finally, an attempt was made to deduce the localization of newly-formed ribonucleic acid by comparison of the ribonuclease treated with untreated autographs. Newly-

formed ribonucleic acid was thus found in liver, kidney, adrenal cortex, and many epithelia. Large amounts were present in tissues where the neoformation of desoxyribonucleic acid occurred, especially in the crypts of the intestine. In contrast, several organs known to be fairly rich in ribonucleic acid, such as pancreas, salivary glands, and thyroid, did not show a significant amount of newly-formed ribonucleic acid.

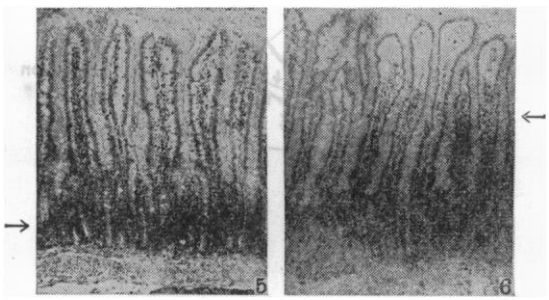

FIG. 3. (5) Duodenum of a rat sacrificed 2 hrs after P^{32} injection. The autographic reaction is located over the cells of the crypts of Lieberkühn, as indicated by the arrow. (6) Duodenum of a rat sacrificed 24 hrs after P^{32} injection. An intense autographic reaction is located over the nuclei in the cells of the villi epithelium; the upper limit of the reaction is indicated by the arrow. A less intense reaction is present in the crypts. (×50.)

Conclusion. Radiophosphorus entering into desoxyribonucleic acid at the time of mitosis may be localized by the "coated autograph" method in tissue sections treated with ribonuclease. The newly-formed desoxyribonucleic acid thus detected is found in the tissues where cell divisions are numerous, e.g. lymphatic and myelogenous tissues, ovarian follicles, intestinal epithelium, etc.

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Protoanemonin as a Mitotic Inhibitor[1]

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Experiments are in progress in this laboratory to determine the effect on root tip mitosis of several drugs and other chemicals. Protoanemonin, $\underset{\_\_\_\_\_}{CH:CH\cdot CO\cdot O\cdot C:CH_2}$, has been found to exert striking effects not only on the nuclei of the meristematic cells but also on the mitochondria and on the structure of the cytoplasm. It has previously been found to be effective as an antibacterial agent (*2*).

Seedlings of *Zea Mays* were grown in beakers lined with moistened filter paper, according to the method of Albaum (*3*), until the roots had reached a length of about 1 cm, and were then transferred to similar beakers in which the filter paper liner was moistened with a solution of protoanemonin. Roots were treated in this way with various concentrations of the drug (10^{-3} M to 10^{-5} M) for various lengths of time (2 hrs to 24 hrs) and then fixed in each of three fixatives: Navashin's for nuclear details, a modified form of Erliki's (*5*) for mitochondria, and a mixture of chromic sulfate, formaldehyde, and copper hydroxide (*4*) for cytoplasmic structure. The root tips were dehydrated by the ethyl-normal butyl alcohol schedule, imbedded in paraffin, sectioned at 8 μ, and stained in iron alum-hematoxylin.

The most striking effect of protoanemonin is the disappearance of mitochondria. The Erliki fixation shows them well in untreated root tips, but not in root tips treated for 24 hrs at 10^{-5} M or higher concentrations or for 2 hrs or longer at 10^{-3} M. Cytoplasmic structure is badly disrupted by treatment at the stronger concentrations and for longer periods, as shown by the chromic sulfate-formaldehyde fixation. In the untreated meristem the image after this fixation is that of an even-textured cytoplasm interrupted by many small, sharply outlined vacuoles. After the longer and stronger treatments the cytoplasm is reduced to irregular strands and darkly staining granules. After shorter treatments and at lower concentrations the only effect is some coalescence of the vacuoles.

The nuclear effect of protoanemonin is strikingly different from that of colchicine. Treatment with the latter drug leads to an abnormally high frequency of metaphases. Treatment with protoanemonin at 2.15×10^{-4} M or higher concentrations for 24 hrs or at 10^{-3} M for 4 hrs or longer reduces the frequency of recognizable mitotic stages to a statistically significant degree. After the longer and stronger treatments, all the nuclei of the root tip are in a condition which resembles interphase or prophase. A small proportion of the nuclei superficially resemble late prophase.

In these nuclei the chromosomes are abnormally contracted, as are colchicine-treated chromosomes, but there is no evidence of chromatid separation or of polyploidy, which are characteristic of colchicine. It appears that protoanemonin exerts its inhibiting effect on mitosis at a different stage in the mitotic cycle than does colchicine.

A more detailed report of these results will be published elsewhere (*1*).

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