of kappa waves above a constant base line occurred during 85% of the choices for one subject; when he merely pressed the key without trying to discriminate, only 17% of the paired tones resulted in this level of activity. Comparable figures for another subject were 36% and 16%.

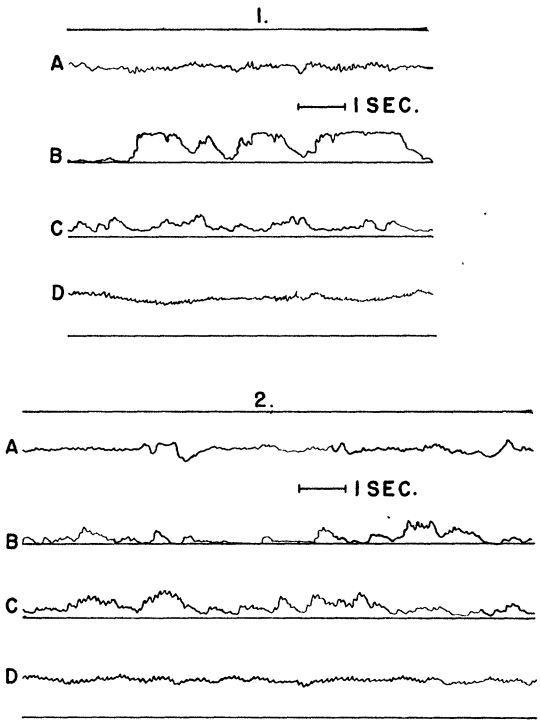

FIG. 4. Part 1, Simultaneous record of kappa and alpha waves during mental multiplication; Part 2, simultaneous record of kappa and alpha waves with "mind a blank."

Out of 31 subjects tested, 18 have shown a recognizable amount of 8- to 12-cycle/sec activity from the canthal placement. Attempts were made to record these waves on subjects not initially exhibiting them by using other electrode placements on the front part of the head. For the most part, these attempts were unsuccessful. Also, the difference in level due to the conditions previously described seems most clear cut for the subjects whose amplitude of kappa activity is highest. Further investigation is required to determine whether kappa waves are actually absent in subjects showing little or no 8- to 12-cycle bursts from the canthal placement. The possibility of such artifacts as poor conduction through the skull and surrounding tissues must be taken into account.

It appears certain from the data available that kappa waves are not directly related to previously described bioelectrical phenomena. Of course, they closely resemble the alpha rhythm in frequency. The conditions for occurrence are, however, different—or perhaps opposite. Alpha waves generally increase in amplitude when the eyes are closed, but kappa waves show no regular differences between conditions of eyes open and eyes closed. Mental arithmetic often inhibits alpha (*4*), whereas kappa waves appear frequently during mental addition or multiplication. Fig. 4, part 1, shows a section of record in which kappa (line A) and alpha (line D) were recorded simultaneously while the subject was doing mental multiplication (eyes fixated). Line B is the accumulation of kappa; line C shows the accumulation of alpha. It is evident that kappa bursts are frequent and are unrelated to alpha activity. Part 2 of Fig. 4 shows a comparable section of record when the subject's eyes are closed and he is trying to "keep his mind a blank." Here, alpha activity is high and kappa low.

The position of the electrodes suggests that the source of kappa bursts may be the temporal lobes of the brain.

The following summary statements may be made:

(1) An intermittent spindle-shaped electroencephalogram with a frequency of 8–12/sec and a maximum amplitude of 20–30 microvolts has been recorded from bipolar electrodes placed just back of the external canthi of the eyes.

(2) These bursts appear to be associated with the process of thinking (discrimination, choice reaction, mental arithmetic, problem solving, etc.).

(3) The bursts are unrelated to previously described alpha activity.

(4) Half of the subjects so far tested exhibit the phenomenon.

(5) It is suggested that the source of the new EEG may be the temporal lobes of the brain.

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Autoradiographs of C^{14} Incorporated in Individual Blood Cells[1]

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Since Altman, *et al.* (*2*) demonstrated that the alpha-carbon atom of glycine labeled with C^{14} is incorporated into the hemin and globin moieties of hemoglobin, it was believed that the incorporated C^{14} in an individual blood cell could be demonstrated by an autoradiograph. To this end a male rat weighing 120 gm was given a

[1] This paper is based on work performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.

total of 3 μc of glycine containing C^{14} in the alpha-carbon atom.[2] The specific activity of this glycine was 1.83 μc/mg. The glycine was administered by means of three intraperitoneal injections of 1 μc each, given at hourly intervals. Blood was taken from the tail veins 25 hrs after the first injection, diluted with serum made from dog blood, and smeared directly on an Eastman NTB emulsion. The smears were dried in air and fixed in methyl alcohol. After an exposure period of 67 days the emulsion plates were developed in Kodak D-19 and cleared, and the cells stained with Wright's stain. The blood smears were made sparsely cellular to insure clear-cut, well-defined autographs and minimal cell clumping.

In order to prove that the autographs are not the result of chemical fogging, similar smears of blood from a control rat were exposed under identical conditions. Details of the technique for preparing blood smears on a photographic emulsion will be described later (*4*).

The accompanying photomicrographs (Fig. 1) show autoradiographs resulting from beta emissions from C^{14} incorporated into blood cells. In Fig. 1, 1, it can be seen that the silver grains are nonuniformly distributed, being concentrated focally about certain individual cells to form autoradiographs. Other cells, such as the erythrocytes in this particular field, produced no autoradiographs. In order to make the autoradiographs prominent at this magnification (×440), the NTB plate from which this photomicrograph was made was developed for a longer time in D-19 than that used for photomicrographs 2, 3, and 4. This procedure enhanced the visibility of the beta radiation effects, but obscured cellular detail. Silver grains between the autoradiographs on the test plates, and in all regions of the control plates, are relatively very small in number per unit area and randomly distributed, as is extraneous background fog.

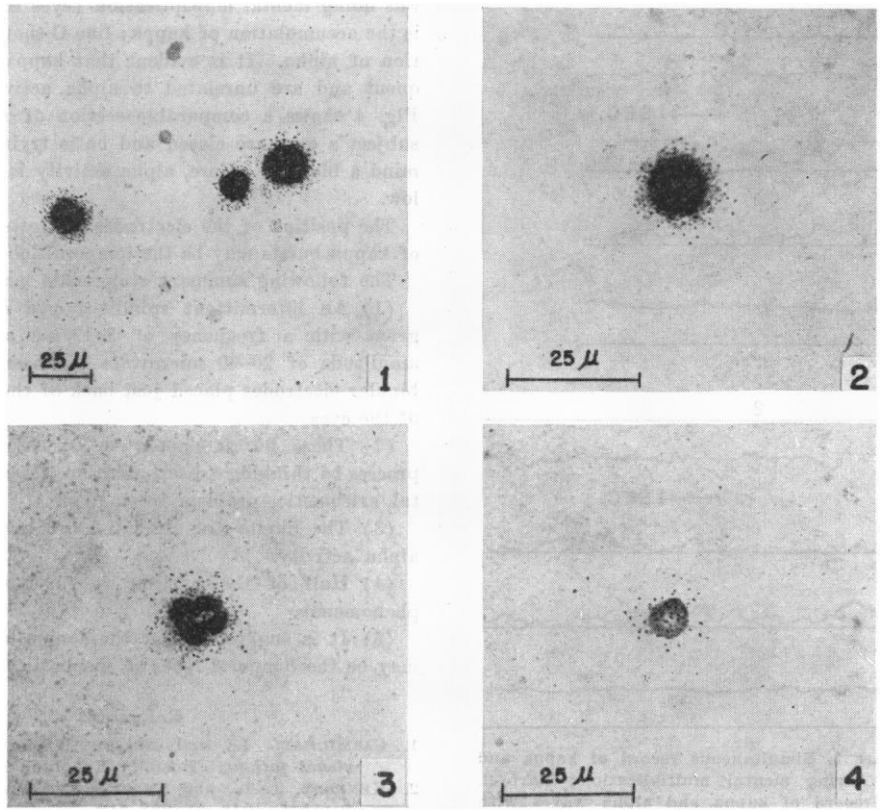

FIG. 1. Autoradiographs of C^{14} incorporated in individual blood cells: (1) Field of test blood smear illustrating nonuniform distribution of silver grains and concentration of grains around certain cells (×440). Cells without autoradiographs are erythrocytes. The exposed test plate from which this photomicrograph was made was soaked in water for 5 min and developed in a dilute solution of D-19 at 20° C for 25 min (D-19 : H_2O = 1 : 3). (2) Lymphocyte (×950). (3) Polymorphonuclear leucocyte (×950). (4) Erythrocyte (×950).
The exposed test plate from which photomicrographs 2, 3, and 4 were made was developed for 2 min in D-19 at 20° C.

The identifiable cells on the test plates include lymphocytes, polymorphonuclear leucocytes, and erythrocytes. Although this technique is not at present completely quantitative, it is apparent that the percentage of cells of each type associated with definite autoradiographs declines in the order: lymphocytes, polymorphonuclear leucocytes, erythrocytes. One possible explanation for this phenomenon lies in the difference in the rate of formation among the three types of cell under consideration. Although the exact life spans of circulating rat blood cells are not known, it is agreed that the erythrocyte has a

[2] This sample of glycine was kindly supplied by B. M. Tolbert, of the University of California.

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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