

### Separation of Flavin and Phosphorylated Flavin

Greene and Black<sup>1</sup> have found that phenol, the cresols, aniline and benzyl-alcohol are suitable for the extraction of free flavin from water.

Using a solution of phosphorylated flavin (purified liver extract), I found that phenol also rapidly took up phosphorylated flavin from water.

It appeared, however, that practically no phosphorylated flavin was extracted by benzyl-alcohol when a solution of phosphorylated flavin in water was shaken with an equal volume of benzyl-alcohol (pH values from 5 to 7.4 and salt concentrations from 0.5 to 4.5 per cent NaCl). Under these conditions one benzyl-alcohol extraction removed 76-79 per cent of free flavin from water. Other aromatic alcohols (phenylethanol, phenylpropanol and phenylethylmethylethylcarbinol also permitted a separation.

Details and applications by these methods of separation (for example, urinary excretion) will be published elsewhere.

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<sup>1</sup> *J. Amer. Chem. Soc.*, **59**, 1820 (1937).

### Biological Effects of Deuteron-Deuteron-Neutrons

THE biological effects of neutrons produced by a cyclotron have been reported by Lawrence and others. However, neutrons generated by a cyclotron, using beryllium targets, are accompanied by a great deal of  $\gamma$ -radiation, and the effect cannot be attributed entirely to neutrons. The purest neutrons ever known are D-D-neutrons, which are produced by bombarding 'heavy' ice ( $D_2O$ ) with deuterons accelerated by high voltage, that is, by the reaction of



Since June 1937, through the kindness of the Nishikawa Laboratory of the Institute of Physical and Chemical Research, Tokyo, I have been examining

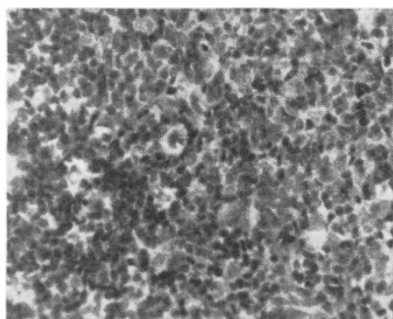


Fig. 1.

IRRADIATED THYMUS.

the biological effects on young rats of irradiating them with D-D-neutrons. As the maximum range of recoil protons generated by neutrons is great, we have made a special ionization chamber with aluminium walls 2 mm. thick, lined with a 0.2 mm. layer of paraffin, and filled with methane gas, in order to measure the total ionization. By comparison with

a known quantity of  $\gamma$ -rays from radium, we measured the intensity of neutrons, and the result showed that, at 250 kv. and 20  $\mu$  A., the ionization at 3.5 cm. from the source was equal to 1.3 r/hour. The following observations were made by irradiating young rats of about 20 gm. body weight with 10-30 r of D-D-neutrons.

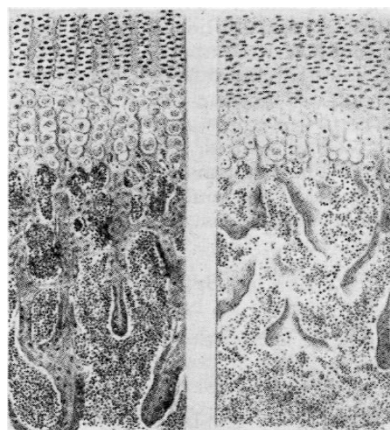


Fig. 2.

BONE AND BONE MARROW BEFORE (LEFT) AND AFTER (RIGHT) IRRADIATION.

The rats, irradiated with 30 r, did not increase in weight, and died in 5-10 days. With 26 r, rats showed no increase in weight for more than two weeks. About one half the amount of neutrons above-mentioned allowed the rats to increase slightly in weight after a week, but they were still very poor in growth, even after four weeks. After irradiation with 12 r, the total leucocytes decreased to about one half, that is, 3,600-4,800. This state continued with or without further irradiation. The most conspicuous was the decrease of lymphocytes, which decreased from 70-80 per cent to 20-40 per cent, and their absolute number was so low as 1000 or less after two weeks. Neutrophil leucocytes, on the other hand, increased from 12-20 per cent to 40-77 per cent, and their absolute number also increased. Monocytes increased for a time, but afterward decreased, and eosinophil leucocytes gradually decreased and finally disappeared. Red cells and haemoglobin gradually decreased, nucleated red cells appearing in the circulating blood in many cases.

At necropsy, anaemia of every organ was marked. Spleen, testis, ovary, thymus, bone, etc., were especially decreased considerably in size. Microscopically, the lymph follicles of the spleen were found to be most quickly affected, where destruction or decrease of lymphoid cells could be seen. Following this, the degeneration of megacaryocytes in pulp came about. Two weeks after exposure to 26-30 r, we found practically no lymphoid cells in the lymph follicles, reticulum cells remained withered in thread-like form, and infiltration of polymorphonuclear leucocytes appeared. Megacaryocytes in pulp having almost gone, many small round myeloid cells with positive oxidase reaction appeared. There were also haemosiderosis and erythrophagia. Four weeks after, many megacaryocytes appeared, also many myeloid cells, showing bloomy myeloid metaplasia, but the regeneration of lymph follicles was still unsatisfactory.