ration of xanthopterin was 50–60 per cent pure. The use of impure xanthopterin may account for recent conflicting reports concerning its role in nutrition.

The effect of ultraviolet irradiation upon the ultraviolet absorption of the vitamin is shown in Fig. 3. The irradiation was carried out with an H-4 lamp without filter for the periods of time indicated. Both the irradiation and corresponding absorption curves were made in aqueous solution (conc. = 0.002 per cent.) at pH 11. It is apparent that the compound is only moderately sensitive to ultraviolet illumination. Our purest specimens of the vitamin have a barely detectable trace of blue-green fluorescence in ultraviolet light. Since this fluorescence increases markedly on exposure to ultraviolet light, we can not be certain that the original very faint trace of fluorescence is not due to a decomposition product. We shall discuss the chemistry and biological activity of the irradiation products in a later paper.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW DIFFERENTIAL STAIN FOR MOUSE PITUITARY[1]

THE need for a reliable and consistent differential stain for the pituitary gland of the mouse in certain experimental work provoked the search for such a method. The literature[2] on the mouse pituitary staining technique was studied and several methods were tried. The technique that was found to be most successful was a combination of several methods rather than any one method previously used. The combination method which has been developed is given in detail below.

(1) Fix tissues in modified Zenker-formol from six to twenty-four hours. Modified Zenker-formol contains only one half the normal amount of corrosive sublimate and one cc less than the normal amount of formalin.

(2) Wash in running water for twenty-four hours and then change to 50 per cent. alcohol for three hours.

(3) Change to 70 per cent. alcohol and leave overnight.

(4) Change to 80 per cent. alcohol for at least two hours or until needed.

(5) Change to 95 per cent. and 100 per cent. alcohols for an hour each.

(6) Change to a 1: 1 mixture of 100 per cent. alcohol and xylol for twenty minutes, to xylol for forty minutes and to a 1: 1 mixture of xylol and paraffin for twenty minutes.

(7) Change to 56°–58° paraffin for one hour and then embed.

The sections of the gland were cut at four micra and were then stained as follows:

(1) Carry sections from xylol through the alcohols to water in the usual manner, using iodine in the 70 per cent. alcohol to remove the corrosive sublimate crystals.

(2) Stain with haematoyxlin 15 to 20 seconds. (Mayer's haematoxylin was used).

(3) Rinse in water and then blue the sections in an aqueous solution of lithium carbonate.

(4) Stain the sections in a solution of picro-orange until they are even yellow. Picro-orange[3] consists of 0.25 gms of Orange G and 100 cc of 80 per cent. alcohol saturated with picric acid.

(5) Rinse in running water until only the red blood cells remain yellow.

(6) Stain in a 1: 1 mixture of 1 per cent. ponceau de xylidine and 0.5 per cent. acid fuchsin as long as the red blood cells remain yellow, about 2 to 5 minutes. (Mcfarlane used ponceau red instead of ponceau de xylidine.)

(7) Rinse in 2 per cent. acetic acid in water.

(8) Differentiate to desired redness in a solution consisting of 40 cc of 95 per cent. alcohol, 20 cc of water and 40 cc of a stock solution. Stock solution includes 25 gms phosphotungstic acid, 2.5 gms picric acid and 100 cc per cent. alcohol.[4]

(9) Rinse in water.

(10) Stain for about 3 minutes in a solution of 100 cc of 2 per cent. Orange G in 1 per cent. phosphomolybdic acid.

(11) Rinse in water and transfer to a 1 per cent. aqueous solution of anilin blue for 15 to 20 minutes.[5]

(12) Rinse in water and dip quickly in 95 per cent. and 100 per cent. alcohols, clear in xylol and mount in balsam.

The technique described above gives results comparable to those of various triple stains on the pituitary glands of other animals. The acidophiles are orange to pink and their nuclei are reddish purple, the basophiles are an intense blue and the nuclei are pur-

[1] This work has been aided by grants from the Commonwealth Fund, Anna Fuller Fund, International Cancer Research Foundation, Jane Coffin Childs Memorial Fund and the National Advisory Cancer Council.

[2] Alexei Koneff, *Stain Tech.*, 13: 2, April, 1938; I. H. Perry and M. S. Lockhead, *Stain Tech.*, 15: 3, July, 1940; Miriam Reed, SCIENCE, 98: 2553; Earl B. Scott, *Stain Tech.*, 15: 2, April, 1940; Aura E. Severinghaus, *Anat. Rec.*, 53: 1, June, 1932.

[3] David McFarlane, *Stain Tech.*, 19: 1, January, 1944.

[4] *Idem.*

[5] C. R. Stockard and E. M. Vicari, *Amer. Anat. Memoirs*, No. 19, Sect. 5, 1941. Detailed suggestions from E. M. Vicari, unpublished material.

ple, and the chromophobes are colorless to very light blue and have purple nuclei.

In using the technique it is best to follow the sections rather closely with the microscope for a while to become accustomed to their appearance at various stages. One advantage of the technique is that if at any time too much stain has been removed the sections can be restained immediately without any further treatment. When using the stains on other tissues less time is necessary in the anilin blue solution.

This method may be used successfully after fixation in a modified Bouin's fluid (using only 1 cc of acetic acid instead of 5 cc) if the sections are mordanted before staining in a 3 per cent. potassium dichromate solution for at least 15 minutes.

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ACTION OF RADIOACTIVE SUBSTANCES ON THE SPEED OF GROWTH OF PENICILLIUM NOTATUM AND THE PRODUCTION OF A POTENT PENICILLIN[1]

WE have based our experiments on the principle that the radiations of minute amounts of radon, or other radioactive substances, have an exciting action on the growth of living substances in contrast with the radiation of larger doses of these radioactive substances which have a destroying power.

Furthermore, a fluorescent substance, for instance, fluorescein, excited by the presence of radioactive substances, emits a light vibration. This light is produced internally in the suspension. The action of small amounts of this light probably has an additional exciting power on living substances.

The radioactive sources used were porcelain tubes filled with emanating radioactive substances (radium). Through the porcelain wall of the tube the radon diffuses. Our cells were prepared for a daily production of about 7 micro curies.

The medium utilized for the culture of *Penicillium notatum* was the usual medium (slightly modified Czapek-Dox medium at PH 6).

For each series of experiments we have studied and compared the growth of the same strain of *Penicillium notatum*,[2] and also the inhibitory power of the penicillin secreted under the following different conditions:

I. Normal growth of *Penicillium notatum* at 24° C.
II. Growth in presence of radon. During the period of irradiation, radon and its short-lived daughter products (Ra A, B, C, C_1, C_2) are present.
III. Growth in presence of radon and a fluorescent substance (fluorescein).

For the control of potency of the penicillin secreted, we have used Heatley's assay method, as described in *Endeavor* of January, 1944. However, we have seeded our plates according to the method described by Thomas, Levine and Vitagliano.[3]

The microorganism tested was a 21-hour culture of *Staphylococcus aureus*.

Under these conditions the Petri dishes examined 24 hours later have shown that the peak of secretion of penicillin, for the irradiated culture, was obtained at least 2 or 3 days before that of the controls.

CONCLUSION

These experiments demonstrate the possibility of substantially decreasing the time required for the growth of *Penicillium notatum* and of obtaining an active penicillin, tested *in vitro,* in the presence of radon and its deposits.

We do not believe that these radioactive substances act through their chemical properties since they are present in negligible quantities. However, it seems reasonable to assert that this action is due to the radiations emitted by these substances. These radiations are Alpha, Beta, Gamma and Delta. As a major part of the radiated energy absorbed by the medium is due to Alpha particles, it seems probable that the observed effect is mainly due to this type of radiation. Experiments are being conducted in order to determine the relative importance in the effect produced by the different types of radiations.

The addition of a fluorescent substance to the medium in which radioactive substances are present improves noticeably the effects of these radioactive elements.

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[2] Received from the Northern Regional Research Laboratory of the U. S. Department of Agriculture.

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