

FLUORESCENCE OF HARDERIAN GLANDS IN MICE OF CANCER-SUSCEPTIBLE AND CANCER-RESISTANT STRAINS¹

IN the course of routine examination of mice under ultra-violet light (G.E. B-H4) it was discovered that there was a great variation in red fluorescence in the exposed Harderian glands of mice of the various strains. The degree of variability may be expressed by the symbols 0, +, ++, +++, +++++. The two extreme variants were found in adult mice beyond 300 days of life of the JK cancer-resistant strain (0-+) and mice of the C₃H strain cancer-susceptible (+++ - +++++). Mice of the inbred strains have shown, so far, a fair degree of constancy, whereas, mice of the NH descent, which are relatively heterozygous (F₅-F₈ generations represented) have shown marked individual variability in red fluorescence of the Harderian glands. An age variation was also observed. Before the eyes were open at 14 days, no fluorescence of orbital contents was detected. In early sexual maturity a high fluorescence was seen. This was found to decrease in intensity in JK mice with advancing age and was completely absent in old mice. This decrease in fluorescence with advancing age was not observed in C₃H mice.

Red fluorescence of the Harderian gland is an indication of the presence of porphyrins (Graffin,² Derrien and Turchini³). Little is known regarding porphyrins within the body. There is, however, some evidence to indicate that they may be involved in the synthesis or destruction of hemoglobin (Lemberg,⁴ Hill and Keilin⁵). Biliverdin results from the oxidative splitting or opening of the porphyrin nucleus of

haemochromogen in the liver (Lemberg⁴). It was found by Strong and Werner⁶ that there was a precocious drop in the hemoglobin level in a mouse of the C₃H strain as compared with one of the JK strain. A similar finding was reported by Strong and Francis⁷ in mice of the A (cancer-susceptible) and CBA (partially cancer-resistant) strains. Strong⁸ suggested that this precocious drop was due to one of two possibilities; (1) that hemoglobin was being produced at a rate lower than normal, or (2) that it was being destroyed at an abnormally high rate. Porphyrins are also known to be important constituent parts of catalase (Zeile^{9, 10} and Stern¹¹) the Pasteur enzyme (Stern and Melnick¹²) and cytochrome c (Hill and Keilin⁵).

Thus it is clear that the present observation may be of interest in the investigation of at least two problems: (1) A genetic analysis of the occurrence and transmission of such a variant, and (2) the investigation of various physiological states as influenced by the presence, absence or abundance of such a chemical within the body. Since mice of the JK and C₃H strains show the maximum degree of difference to both cancer susceptibility and porphyrins in the Harderian gland, such an investigation should include a search for a possible relationship between porphyrins and some physiological process that may be correlated with carcinoma susceptibility.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A NEW DIGESTION TUBE

THE chemist is often faced with the problem of having to determine accurately, by means of acid digestion, small quantities of protein. During digestion, these protein solutions may bump and foam. The possible loss of material by bumping may usually be controlled by good technique. Occasionally, however, with the most careful technique, loss of material may invalidate the entire procedure.

Certain types of determinations can be effected by

the use of the N.P.N. tube and the Kjeldahl flask, to mention but two of the several digestion tubes and flasks available to chemists. Few of the methods involving the use of the N.P.N. tube are accurate.

⁵ R. Hill and D. Keilin, *Proc. Roy. Soc. London*, 107: 286-92, 1930.

⁶ L. C. Strong and T. H. Werner, *Am. Jour. Cancer*, 26: 767-69, 1936; 27: 115-19, 1936.

⁷ L. C. Strong and L. D. Francis, *Arch. Path.*, 23: 202-06, 1937.

⁸ L. C. Strong, *Am. Jour. Cancer*, 27: 500-09, 1936.

⁹ K. Zeile and H. Hellström, *Hoppe-Seyler's Zeits. für Phys. Chem.*, 192: 171-92, 1930.

¹⁰ K. Zeile, *Hoppe-Seyler's Zeits. für Phys. Chem.*, 195: 39-48, 1931.

¹¹ K. G. Stern, *Jour. Biol. Chem.*, 112: 661-, 1936.

¹² K. G. Stern and J. L. Melnick, *Jour. Biol. Chem.*, 139: 301-23, 1941.

¹³ Rockefeller Foundation fellow at Yale University, 1940-41.

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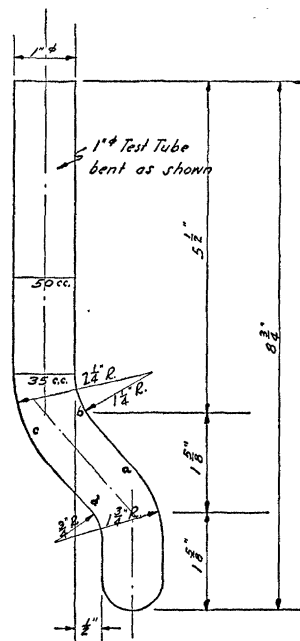
² A. I. Graffin, *Anat. Rec.*, 79: suppl. 25, 1941 (abstract).

³ E. Derrien and J. Turchini, *Compte rend. Soc. de Biol.*, 92: 1028-29, 1925.

⁴ R. Lemberg, *Biochem. Jour.*, 29: 1322, 1935.

This is especially true of the protein determination of cerebrospinal fluid, a process associated with so much foaming and bumping that, for clinical purposes, the results are often not as acceptable as they should be. The Kjeldahl method is exact, but its use necessitates a nitrogen distillation system and accurate burettes for back-titrations. The apparatus is expensive and the technique time-consuming.

We have designed and constructed a new digestion tube by means of which the loss of material due to foaming and bumping can be eliminated. The tube



DIGESTION TUBE
FIG. 1

is designed so that a drop of boiling solution can not be shot directly outwards. When thrown upwards, it must necessarily strike the wall of the tube along the lines "a" and "b" and flow back into the solution. Foam also will rise up between the points "a" and "b" and descend by points "c" and "d." Seldom does it rise beyond the point "b."

By the use of the tube described, the more difficult types of digestion can be performed with ease and accuracy. There is no loss of solution and much saving of time. The most ordinary technique will furnish exact results. The new digestion tube is especially suitable for those methods for which the N.P.N. tube is, at present, used.

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A TECHNIQUE FOR CONTINUOUS OR INTERMITTENT OBSERVATION OF THE CONTRACTILE VACUOLES OF THE PARAMECIUM

THE methods commonly used for demonstrating and studying the contractile vacuoles of *Paramecium* have the serious disadvantage of not permitting the observer to view the animals intermittently over a period of several days. In the technique described below, continuous observation is made possible by studying the animals on the surfaces of agar plates.

PREPARATION AND USE OF THE AGAR PLATES

Filter 1,000 cc of culture medium which has supported a vigorous growth of *Paramecium* through coarse filter paper. The medium should be clear and transparent. Bring the filtrate to a boil and add, while stirring, 10 gms of agar-agar. Boil slowly until all the agar is dissolved. The agar is then poured, while hot, into Syracuse watch glasses to a depth of approximately 5 mm. The agar is then allowed to cool, without agitation, until it is firmly set.

When the agar is set, one drop of a rich culture of *Paramecium* is placed in the center of the dish, which is then tilted from side to side to spread the drop. Within a few minutes enough water has evaporated from the surface to impede locomotion. The animals may then be studied at leisure.

For prolonged observation of the same preparation it is necessary to prevent undue evaporation from the surface of the agar. This is best accomplished by inverting the dish over another watch glass partly filled with water. In this way we have kept and observed animals in the same preparation for as long as ten days. Temporary preparations may be made with distilled water, but the animals do not survive on the surface of agar so prepared.

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BOOKS RECEIVED

- CHAMPION, F. C. *University Physics; Light*. (Part Three of a Five-part Course.) Pp. 172. Illustrated. Interscience Publishers. \$1.50.
- COHEN, I. BERNARD, Editor. *Benjamin Franklin's Experiments*. Pp. xxviii + 451. Illustrated. Harvard University Press. \$4.00.
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- YATES, RAYMOND F. *Science Calls to Youth*. Pp. xiii + 205. Illustrated. Appleton-Century. \$2.00.