standard solution 25 per cent greater than that used to produce the two preceding standard contractions. Measure the height of each of the five contractions. The first four contractions are to be considered submaximal and equivalent if the difference in height between the highest and lowest of these four is less than half the difference in height between the lowest of the four and the contraction resulting from the increased dose of standard. Two such series of five contractions shall constitute an assay.

We wish to emphasize the fact that the record of an assay obtained in this way furnishes proof of the submaximal character of the first four contractions. Furthermore the definition makes clear exactly what is required by the term equivalent as used in regard to these four contractions.

It is further suggested that the wording in the rubric in regard to potency should be changed from "One cc. of Solution of Posterior Pituitary produces an activity upon the isolated uterus of the virgin guinea pig corresponding to not less than 80 per cent and not more than 120 per cent of that produced by 0.005 Gm. of the Standard Powdered Posterior Pituitary" to the following wording: "One cc. Solution of Posterior Pituitary produces an activity corresponding to that produced by 0.005 Gm. of the Standard Powdered Posterior Pituitary." A note worded as follows should then be added at the end of the assay procedure: "*Note*—Owing to the many variable factors in the assay of solution of posterior pituitary, evidence of potency in all assays of solution of posterior pituitary to within 20 per cent above or below the standard, is acceptable."

Further study of the problem of the assay of posterior pituitary extracts is now under way in this laboratory. Among the points under consideration are the following: (*a*) the composition of the bathing fluid; and (*b*) alternative or subsidiary methods of assay, namely, the pressor assay for the pressor principle using the anesthetized dog (3); a modification thereof using the anesthetized cat; and the blood pressure depressor assay of Coon (4) for the oxytocic principle using the anesthetized chicken. This latter method has been shown to be simple, speedy and reliable, and devoid of the many technical difficulties involved in the present official method.

SUMMARY

1. A refinement of the definition of what constitutes an official assay of solution of posterior pituitary is proposed.

2. The results of a coöperative study of the proposed definition carried out by six laboratories testify to its reliability and practicability.

3. A change is recommended in the wording of the rubric in regard to potency of solution of posterior pituitary.

4. A plan of future work on posterior pituitary assay is outlined.

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# Laboratory Apparatus and Procedure for Preparing Permanent Records of Biological Vitamin D Assays*

*By Arthur D. Holmes, Madeleine G. Pigott and Arthur N. Terry*

INTRODUCTION

The United States Pharmacopœia (1) supplies detailed information for conducting the official biological assay of cod liver oil and related substances for vitamin D. However, no suggestions are offered for preparing permanent records of the results of vitamin D assays. Since the results of the assays should be available for considerable periods of time subsequent to the conclusion of the assay, it is essential that these results be preserved in a permanent form, as free as possible from the personal factor of the assayist. Obviously, whenever there is a possibility of the results of vitamin D assays being used in a legal controversy or in court action their availability in a permanent form is of supreme importance. Accordingly, it seems desirable to describe the procedure and equipment used in this laboratory for preparing permanent records of vitamin D assays both to assist others who are conducting such assays and to assist, particularly, those who are just establishing vitamin D assay procedures.

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## APPARATUS AND PROCEDURE

At the conclusion of the U. S. P. "assay period" (1) the experimental rats are killed by anesthetizing with chloroform. A lethal chamber (Fig. 1) 14 x

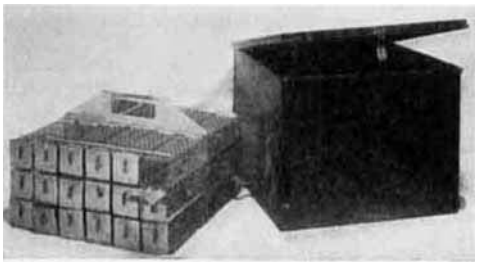

Fig. 1.—Lethal Chamber.

14 x 12 inches, outside dimensions, provides for anesthetizing thirty-six rats simultaneously. It is made of 22-gage galvanized iron, thoroughly soldered on all seams. The upper edge of the sides are rolled over a $^3/_{16}$-inch iron rod. The cover, which is carefully fitted to the chamber, is made rigid by having its lower edges rolled over a $^3/_{16}$-inch iron rod. It is hinged to the chamber in the back and the clasp in front provides a tight closure. An assembly of thirty-six compartments $6^1/_2$ x 2 x 2 inches made out of perforated 22-gage galvanized iron containing the maximum number of $^5/_{16}$-inch holes (machine shop scrap) are firmly attached to each other by being soldered to $^3/_{16}$-inch rods. The assembly has a convenient handle for moving the entire group of compartments as a unit. Obviously the assembly may contain more or less compartments depending upon the requirements of the laboratory for which it is being made, but any variation in the size of the assembly of compartments will necessitate changing the size of the outside portion of the lethal chamber.

Fig. 2.—Equipment for Staining and Developing Tibiæ.

The doors for the compartments are made of 26-gage galvanized iron 2 inches wide, with the lower edge having two extending strips which are doubled back and soldered to provide hinges. An extended upper edge, bent at right angles to the cover, serves as a friction clasp for holding the door closed. Three links of plumbers No. 000 safety chain are soldered to each cover to serve as a handle for opening it. The assembly of thirty-six compartments is supported in the chamber by a $^3/_4$-inch ledge soldered to the inner side of the walls of the chamber 2 inches from the bottom. This provides free circulation of chloroform fumes throughout the chamber from the chloroform placed at the bottom of the chamber. As the experimental animals are placed in the lethal chamber the identification of each is placed within the compartment or noted upon the door of the compartment containing the animal in question.

When the anesthetized animals are removed from the lethal chamber both tibiæ are dissected, completely freed of all adhering tissue and placed in vials containing 10% formaldehyde solution. The dimensions of the vials (Fig. 3) are 60 x $16^1/_2$ mm. or approximately 8 cc. capacity. After the tibiæ have remained in the formaldehyde for at least twenty-four hours the right tibia is removed, washed and split longitudinally (the left tibia is retained in the formaldehyde for future use if necessary).

The proximal end of the right tibia is washed and rinsed in distilled water and immediately immersed in a 2% aqueous solution of silver nitrate for one minute. The section is rinsed with water, immersed in distilled water and exposed to light from a 200-watt Mazda lamp at a distance of about $5^1/_2$ inches. The sections remain exposed until the calcified area develops a clear, well-defined stain when they are again thoroughly rinsed in water and immersed in a 5% solution of sodium thiosulfate for about 2 minutes. After washing in water the extent of calcification of the rachitic metaphysis of each section is then recorded and the section is returned to the original specimen bottle and is retained for photographing and any possible future reference.

In order to stain and develop a number of tibiæ at the same time the monel metal tray (Fig. 2) was

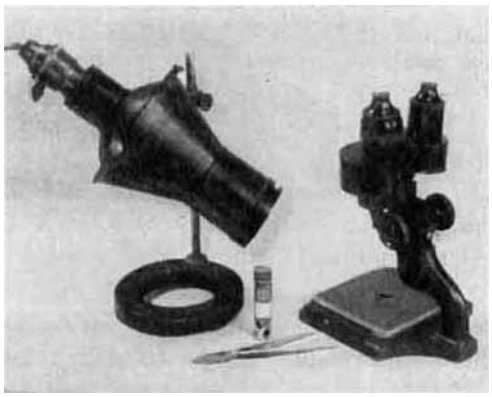

Fig. 3.—Equipment for Examining Tibiæ.

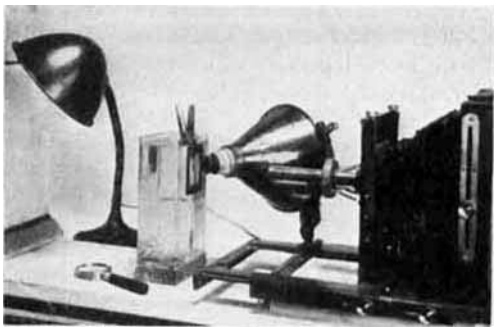

Fig. 4.—Equipment for Photographing Tibiæ.

manufactured. It consists of solid sides, a bottom of 1/8-inch mesh aluminum wire and partitions which produce 24 compartments 3/4 x 3/4 inch. The metal tray with the tibiæ properly identified is placed alternately in photographic glass trays containing the solutions required for the development of the tibiæ. While the tibiæ are being exposed to the action of light the wooden form (Fig. 2) containing the 200-watt Mazda lamp is superimposed on the glass and the metal tray containing the tibiæ in distilled water. Since the tray with the tibiæ can be placed at a predetermined distance from the lamp and can be exposed for a desired period of time, all of the tibiæ to be developed on any given day can be handled under uniform conditions.

The degree of healing or recalcification of the tibiæ is viewed, through a Spencer Universal Binocular microscope (Fig. 3) using appropriate magnification. Ordinarily a magnification of 21 times is found desirable. While the tibia is being examined it is illuminated with a Spencer daylight lamp containing a 60-watt Mazda lamp. The degree of healing or recalcification of the tibia is reported as being one, two, three or four "+." This is an empirical unit which has been assigned value by Bills, Honeywell, Wirick and Nussmeier (2). Obviously, the estimated degree of healing or recalcification of the tibiæ in terms of the empirical unit "+" will vary with the experience and judgment of the assayist. Hence it is highly desirable that accurate photographs of the tibiæ should be made at the conclusion of the assay period and that these be preserved in good condition for future use. Such photographs are of inestimable value when the results of vitamin D assays become involved in legal controversy or court procedures.

The photographic records of the calcification of the tibiæ were produced with a simple and inexpensive equipment (Fig. 4) assembled in this laboratory. An ordinary camera with ground glass back and a plate holder for holding 3 1/4 x 4 1/4-inch plate is fitted with a cardboard duplicator which divides the plate, when in horizontal position, for two exposures on each plate. A low power Bausch & Lomb No. 10 microscope is attached to a wide angle camera lense. The short focus of the wide angle lense helps to make the equipment more rigid and the wide angle, with iris diaphragm, allows the use of different stops. To prevent the rat tibia from dry-

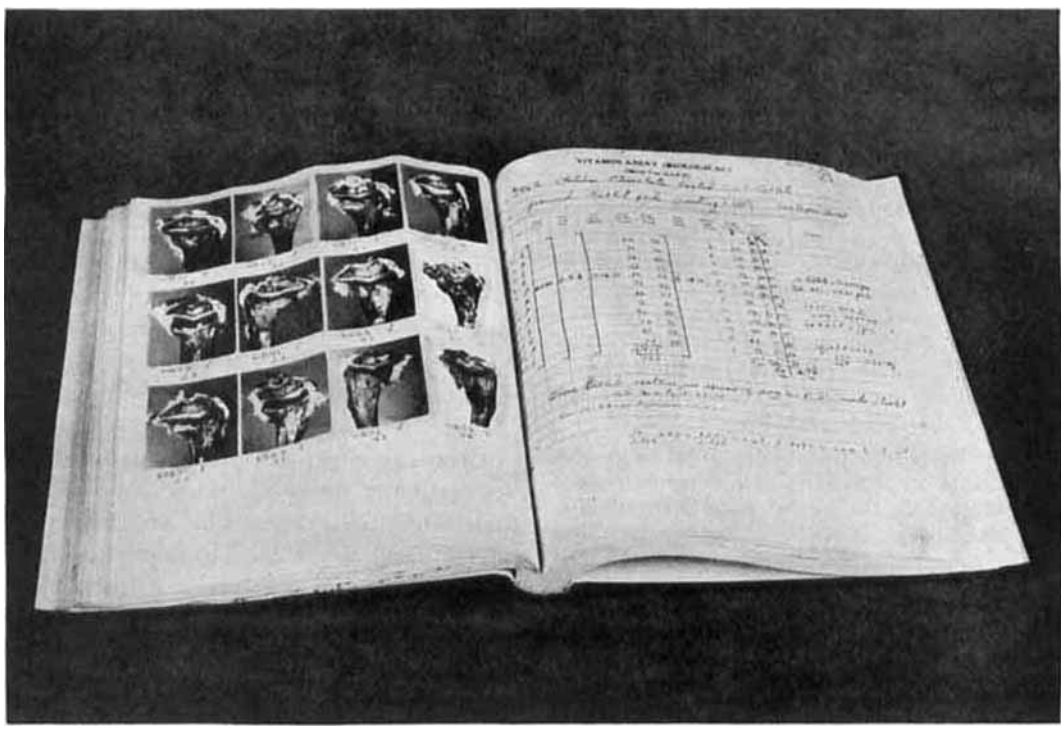

Fig. 5.—Book for Permanent Records.

ing during photographing and to prevent reflections from the surface of the bone, it is suspended (with spring clothes pin) in a battery jar of water. Scotch tape attached to the outside of the battery jar facilitates in the placing of the tibia in proper alignment with the camera. While the tibia is being photographed it is illuminated by a Photoflood Bulb No. 1 in an ordinary reading lamp equipped with a metal cone for converging the light directly upon the tibia. The "exposure time" for making the photographs is materially shortened when a low power magnifying lense is installed in the opening of the metal cone. However such a magnifying lense is not a necessary part of the photographic equipment.

Trials with various types of backgrounds have proved that the most satisfactory results are obtained, when the background is ground glass thoroughly illuminated with a 60-watt Mazda lamp. The distance from the end of lense combination to face of battery jar is $3^3/_4$ inches, from photoflood bulb to specimen is 8 inches, from background to specimen 12 inches and the distance from the ground glass background to the lamp illuminating it will be varied according to conditions, principal of which is the degree of illumination of the room in which the photographs are made.

For rapid quantity production the camera is not focused for each exposure but is adjusted so that it will turn from side to side. It is focused for the first tibia on the center of the plate, the cardboard duplicator is then inserted in back of camera. The camera may then be turned from side to side and the image of the bone will be in the correct location for photographing on one half of the $3^1/_4$ x $4^1/_4$-inch plate. After an exposure is made on one half of the plate the duplicator is slid over and the camera is turned so that image is on the other half of plate where a second exposure is made. The Wratten and Wainwright regular Panchromatic plates seem to be preferable and for best results the plate is over exposed and under developed. By slightly over exposing the plate greater detail is obtained in the shadows and dark portions of the tibiæ. Then by under developing slightly, extreme contrast in the high lights can be avoided. For a well-stained specimen the average exposure is 25 seconds with lense stop at F32 and the negative is developed for about $1^1/_2$ minutes. The prints of the tibia are made on glossy contact single weight paper but this is optional with the photographer.

When the photographs of the tibiæ have been completed they are mounted in a permanent record book (Fig. 5). The record book illustrated is specially prepared in this laboratory by assembling alternately sheets of plain Bond paper and printed forms for recording vitamin D assay results and having them permanently bound. From a legal aspect this type of record book has very decided advantages over a loose-leaf type of record book since there can be no possibility of the data having been changed at any time subsequent to the initial recording of it.

The vitamin D assay results which appear on the right of the record book comprise data concerning the identification of the sample, the dilution at which it was fed, data for each experimental animal relative to its identification number, sex, its initial, final and increase in weight, relative to the amount of the sample consumed daily, the dates for the beginning and ending of the assay period, the amount of ration consumed, the extent of the healing of the tibiæ and remarks.

The photographs of the tibiæ are mounted on the left-hand page of the record book facing the vitamin chart and definite attention must be given to the type of adhesive used for mounting the photographs. Formerly a rubber cement type of adhesive was used in this laboratory because of its excellent adhesive qualities. Unfortunately with the passage of time the photographs became badly discolored due, presumably, to interaction between the "rubber cement" and materials used in preparing the photographs. At present "Foto-Flat" appears to be the best type of adhesive for permanently mounting photographs of tibiæ.

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(2) Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M., *J. Biol. Chem.*, 90 (1931), 619.

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# Salts of Alkaloids with Bromocomplexes of Some Heavy Metals*

*By E. P. White*

### INTRODUCTION

It has been found by Meurice (1) that dilute brucine sulfate solution and potassium bromide with a trace of cadmium salt produce separation of a white double bromide, while copper, aluminum, iron, chromium and chloride give no precipitate. Preliminary experiments suggested that a detailed investigation of the precipitates formed under these conditions would be of theoretical interest, and might lead to microanalytical reactions of value. To our knowledge salts of this series have not been analyzed formerly nor their properties described. After this

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