non-specific irritative effect of the inoculum.

No evidence for an increased resistance attributable to the vaccination was obtained. The incidence of all types of upper respiratory disease during the 2-month period succeeding vaccination in both inoculated and uninoculated groups did not differ significantly. Several explanations may be offered for the ineffectiveness of the vaccine. The strains of Influenza A may have differed in antigenic composition from the prevailing epidemic strain. This possibility has been invoked to explain the relative ineffectiveness of vaccination in other parts of the country during the epidemic of last spring.[12,13,14] The quantity of vaccine administered may have been too small to establish resistance. Vaccination may have been undertaken too late, since serologic evidence was obtained that Influenza A was present among the hospital personnel within ten days following the administration of the vaccine.

Although the prophylactic value of intradermal vaccination has not been demonstrated, additional experiments would seem desirable in view of the results of Van Gelder and his co-workers and those reported in this communication.

12 Francis, T., Jr., Salk, J. E., and Quilligan, J. J., *Am. J. Public Health*, 1947, **37**, 1013.

13 Sigel, M. M., Shaffer, F. W., and Henle, W., *J. Bact.*, 1947, **54**, 277.

14 Smadel, J. E., *Bull. U. S. Army Med. Dept.*, 1947, **7**, 795.

Summary. 1. Intradermal inoculation into 39 adults of 0.02 ml of concentrated Influenza A and B vaccine gave rise to a mean antibody response (antihemagglutinin) against Influenza B virus which approached that obtained by others following the subcutaneous injection of 1.0 ml of undiluted vaccine. A greater mean increase in antibody against Influenza A was observed in the same group, although this increase could not be attributed conclusively to the effect of the vaccine.

2. The intradermal injection of 0.02 ml of concentrated vaccine into 449 adults was followed within 20 minutes by a local erythematous reaction exceeding 10 mm in diameter in 373 or 83.1%. In 383 or 85.3% of the same group delayed local erythematous reactions exceeding 10 mm in diameter were present after approximately 48 hours accompanied in many cases by slight induration.

3. No correlation could be established between the intensity of the immediate or delayed dermal reaction and the level of antihemagglutinin in the blood.

4. Mild systemic reactions were reported by 75 (16.7%) of 449 vaccinated persons.

5. Although cases of Influenza A infection were demonstrated in the community shortly after vaccination, the rate of all upper respiratory disease during the succeeding 2-month period in a group of 316 vaccinated persons was not significantly different from the rate among 329 unvaccinated persons working in the same institution.

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Use of the Spectrophotometer for Measuring Melanin Dispersion in the Frog.

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It is well established that melanophore changes in the frog are related to environmental conditions as well as endocrine factors within the animal. However, there apparently is no satisfactory data on how completely a dispersion or concentration of melanin in the skin will interfere with the reflectance of light by the other pigments present.

A review of the literature regarding methods for measuring melanin dispersion or concentration in the skin indicates that most of the observations made were patterned after

the general descriptive technic reviewed by Parker[1] in which the individual melanophores are given a numerical value corresponding to the various stages of expansion they may happen to be in at the time of examination, and which neglects to consider how effectively the contracted or expanded state of these melanophores will change the appearance of the frog or rest of the pigment normally present in the skin. It should also be pointed out that, even though the results obtained by this method for recording melanin dispersion or concentration in individual melanophores may be accurate and consistent, there always remains an unavoidable subjective factor when one attempts to catalogue melanophores in different stages of expansion in a microscopic field. It would therefore be highly desirable to have an objective method which would give quantitative information concerning the actual amount of masking the dilated melanophores will have over the rest of the skin pigments in the intact animal.

Although Hill and Solandt[2] and Smith,[3] by attaching a photo-electric cell to a microscope, have recorded the variation in transmitted light through the skin and scales of fish under dark and light adapted conditions, they made no attempt to study the effects of melanin dispersion in the intact skin of the frog on the light reflected at each wave length of the visible spectrum. In a search for a method that would answer the requirement above mentioned, it was found that the Hardy[4] photo-electric recording spectrophotometer would successfully give information on the kinds of pigments giving rise to the color of the skin as well as give an objective measurement of the amount of reflectance each pigment may give at its respective wave length when the melanophores are in an expanded or contracted state. The results obtained from experimentation with this technic showed significant differences in skin reflectance in frogs that had previously been either light or dark adapted, and we herewith present the experimental technic used and the results obtained.

In preparing frogs for this type of recording 2 different sets of experimental conditions were used: one group of 8 frogs was exposed to light, and an equal number to dark for the same period of time. To accomplish light adaptation, the frogs were kept for a minimum of 72 hours in a white container which was illuminated at all times by two 40-watt bulbs; for dark adaptation the frogs were kept in a black container for 72 hours, also illuminated by two 40-watt bulbs. To record the skin reflectance the frogs, unanesthetized, were made immobile by fastening them to a frog board. They were then placed so that the back of the frog covered the round aperture (one inch in diameter) of the recording spectrophotometer. The light rays of various wave lengths of the visible spectrum were individually focused on the skin of the frog, and the amount of light reflected in each of the wave lengths was measured and recorded on ruled paper on a percentage reflectance basis. The spectrophotometer was moved by hand in preference to having the complete rotation of the drum made by the motor. The total time necessary for making a complete record was one minute, whereas, with the motor, a total time of 3 minutes was necessary. To insure accuracy the frogs were always placed in the same position before the light aperture of the instrument and the time of exposure was kept constant to avoid any inconsistencies so far as light from the spectrophotometer and melanophore responses were concerned.

The reflectance from the skin surface of the light adapted frogs is definitely greater in all wave lengths measured than the reflectance from the skin of the dark adapted frogs. This can be seen in the graph, which shows the average % reflectance at various wave lengths for light (upper curve) and dark (lower curve) adapted frogs. The results also indicate that melanophore expansion is apparently able to mask the reflecting power of the other pigments in the frog skin. It is

[1] Parker, G. H., *Biol. Bull.*, 1943, **84**, 273.

[2] Hill, A. V., and Solandt, D. Y., *J. Physiol.*, 1934, **83**, 13.

[3] Smith, D. C., *J. Cell. and Comp. Physiol.*, 1936, **8**, 83.

[4] Hardy, A. C., *J. Optic. Soc. America*, 1935, **25**, 305.

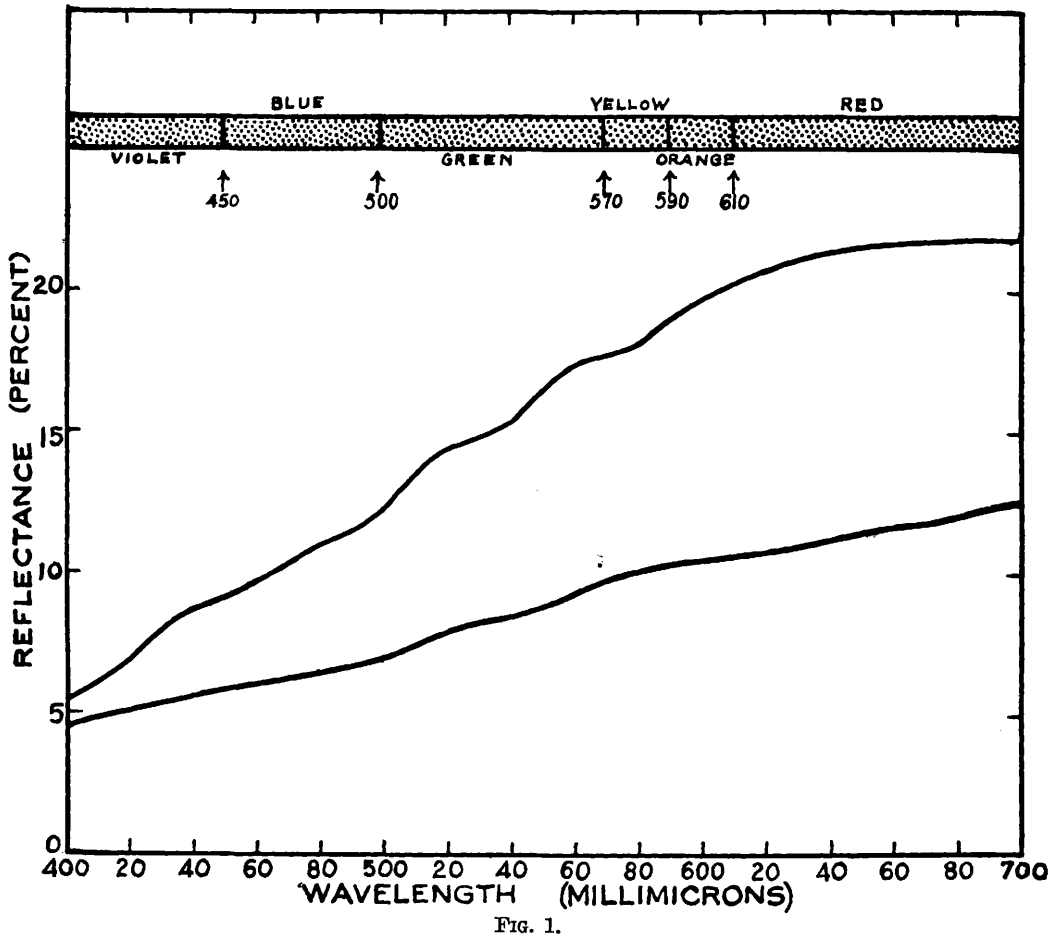

FIG. 1.

interesting to note that the general shape of the curve and degree of reflectance from the skin of the frog under these conditions compare quite closely with the degree of melanin distribution and concentration obtained by Edwards and Duntley[5] from the dorsal surface of the hand of a Negro when examined with the same technic as used in these experiments. Furthermore, the reflectance from the skin surface of the frog is greater for wave lengths at the red end of the spectrum than at the violet end. Statistical analysis of the data justifies these conclusions. Although it is obvious from the data that there is a definite difference in reflectance between light and dark adapted frogs, it is rather surprising to find that the light adapted frogs reflect an average of only 21.8% in the red end of the spectrum where the actual reflectance is at its maximum, while the dark adapted frogs, black as they appear to the eye in comparison, reflect an average of 12.3% at the same wave length. Surely these quantitative measurements show the inadequacy of our subjective observations.

In a few additional experiments, it was interesting to note that frogs made permanently pale as a result of complete hypophysectomy[6] showed melanophores that did not constrict so much or allow for so great a reflectance as those in frogs that were light adapted. If the frogs were made permanently dark because of melanophore expansion following injury of the pars tuberalis region of

[5] Edwards, E. A., and Duntley, S. Q., *Am. J. Anat.*, 1939, **65**, 1.

[6] Hogben, L. T., *Quart. J. Exp. Physiol.*, 1923, **13**, 177.

the pituitary gland,[7] the dilated state of the melanophores was greater and showed definitely less reflectance than frogs that were dark adapted by environmental stimulation only. Further studies of the same kind in which various dosages of related hormones are given may lead to helpful information concerning the mechanism of chromatophore action in animals.

Summary. (1) The spectrophotometer has been used as an instrument for recording the effects of melanophore expansion on the reflecting power of the skin pigments of the frog. (2) The reflectance in the light adapted frogs may be as much as 10% above that of the dark adapted frog at the same wave length. (3) Melanophore expansion in dark adapted frogs masks the reflecting power of the skin pigment at the red end of the spectrum more than at the violet. (4) The average skin reflectance in the light adapted frogs is not more than 21.8%. (5) A difference between the response of melanophores to environmental conditions and operations of the hypophysis is suggested.

[7] Steggerda, F. R., and Soderwall, A. L., *J. Cell. and Comp. Physiol.*, 1939, **13**, 31.

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Interrelationship of Vitamin D and the Sex Hormones in Calcium and Phosphorus Metabolism of Rats.

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Introduction. The present therapeutic trend for treating chronic arthritis in humans involves the use of high dosages of vitamin D, and has many successes to its credit.[1,2] The favorable treatment of Raynaud's disease in the same manner, in conjunction with sex hormones, has been reported.[3] The high incidence of arthritis in women at a time near to or following the menopause, and the more gradual appearance of this disease in men, is well known. This leads to the suggestion that the role of the sex hormones in these relationships involving the therapeutic use of vitamin D should be more fully understood.

Hypertrophy of the parathyroids has been described in a number of suggestively analogous conditions, viz.: rickets,[4] scleroderma,[5,6,7] Raynaud's disease,[8,9] and chronic arthritis.[10] Experimentally, by perfusion experiments,[11] a low calcium level in the perfusate leads to similar hypertrophy which is overcome by the addition of adequate amounts of calcium. Hyperphosphatemia also induces a corresponding enlargement as one would expect. Vitamin D reduces the compensatory hypertrophy induced by low calcium feedings.[12]

[1] Snyder, R. Garfield, Squires, Willard, Forster, John, Traefer, Cornelius, and Wagner, Lewis Clark, *Indust. Med.*, 1942, **11**, 295.

[2] Wagner, Lewis, *Industr. Med.*, 1942, **11**, 313.

[3] Norman, G. F., *West. J. Surg., Ob. and Gyn.*, 1938, **46**, 553.

[4] Nonidez, J. F., and Goodale, H. D., *Am. J. Anat.*, 1927, **38**, 319.

[5] Cornbleet, T., and Struck, N. C., *Arch. Dermat. and Syph.*, 1937, **35**, 188.

[6] Leriche, R., and Jung, A., *La Presse Med.*, 1938, **46**, 809.

[7] Leriche, R., *Gazette du Hospitaux*, 1936, **109**, 209; Ab. *J. A. M. A.*, 1936, **106**, 1214.

[8] Bernheim, Alice R., *J. A. M. A.*, 1933, **100**, 1001.

[9] Bernheim, Alice, and Garlock, J. H., *Ann. Surgery*, 1935, **101**, 1012.

[10] Wootton, W. T., *J. Missouri M. A.*, 1936, **33**, 129; Abs. *J. A. M. A.*, 1936, **106**, 1854.

[11] Path, Harvey M., Wallerstein, Elizabeth, and Luckhardt, Arno B., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 580.

[12] Carnes, William H., Pappenheimer, Alvin M., and Stoerk, Herbert C., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 314.