mgs, as compared to 27.3 mg for the testes of a male autopsied in January of the same year. The ovaries weighed 126.8 and 110.3 mgs, as compared to 56.7 mg for the ovaries of a bird killed in mid-winter. The injections of 5 to 10 grams equivalent of an alkaline hypophyseal[6] extract in 3 male grouse for 10 days resulted in testes weighing 238.2, 206.4 and 179.4 mgs, an increase of approximately five times.

Similar results were found in the pheasants. The testes of 3 male pheasants autopsied on August 3, 1936, three weeks after the close of the breeding season, weighed 367.8, 617.1 and 612.5 mgs. The testes of birds in the winter weighed from 69.7 to 164 mgs, while in full sexual activity they weighed over 6 grams. Three male pheasants, each receiving a pyridine extract of sheep hypophysis equivalent to 5 grams of dried glands for a period of 10 days, yielded testes weighing 1,232, 2,209 and 3,001 mgs, as compared to 907 and 1,052 mgs for two uninjected controls. The difference in weight of the testes of the controls and those autopsied August 3 is due to the fact that they belong to different age groups. The average increase was 119 per cent.

In both grouse and pheasant, histological examination of the testes confirmed the gross observations. In all injected birds, the testes showed mitotic activity, an increase in the number of cells in the germinal epithelium and an enlargement of the tubules. Normal sperm were found in the largest testes of the injected pheasant; none were found in the controls. The control testes of both pheasant and grouse gave evidence of degenerating to the resting condition, the grouse testis having regressed further.

(4) The gonads of immature pheasants will respond to adequate hypophyseal stimulation. A group of 8-week-old male pheasants were given a similar amount of the sheep hypophyseal extract for the same period as the adult pheasants. The testes of the young injected birds weighed 197.0, 143.3 and 93.2 mgs, and the testes of the controls weighed 61.2, 45.2 and 67.8 mgs, an average increase of 148 per cent. Microscopically, the stimulated testes were easily distinguished by the enlargement of the tubules and the increased number of division figures in the germinal epithelium.

SUMMARY

The results of these experiments has led us to conclude that light is a primary factor in inducing sexual activity in pheasants, quail and grouse, through the mediation of the hypophysis. Once this hypophyseal-gonad reaction is started it can not continue indefinitely with adequate light but only until the hypophysis falters in the production of the gonad-stimulating hormone through causes unknown. The immature pheasant resembles the adult at the close of the breeding season in that the gonads are capable of responding to adequate hypophyseal stimulation.[7]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

THE PHOTOELECTRIC DETERMINATION OF PHOSPHORUS IN ESTUARINE WATERS

IN estuarine waters there is much variation in salinity and heavy metal content, both of which factors may interfere with phosphorus determination, when the Denigès method is followed. Errors due to such factors must be taken into consideration. Also, the blue color of the reduced phospho-molybdate, as produced in the Denigès method, may be of improper magnitude (intensity and quality) for comparison with a standard blue solution by means of an ordinary comparison colorimeter. Accordingly, a photoelectric colorimeter was devised to study the color production.

A Weston photometer measuring light intensity in foot candles, a ruby glass filter, a pyrex tube 30 cm long closed at both ends to hold the solution and a Spencer microscope lamp served as the photoelectric colorimeter. The solution to be tested, which was blue in color after being treated according to the Denigès method, was put into the 30 cm pyrex tube, in the ends of which plane glass pyrex windows were fused. An opening left on the side of the tube served for filling and emptying. By placing such a cylinder, filled with the blue solution to be tested, in the path of the beam of light, the absorption of the light by the solution expressed in terms of change in light intensity would be measured by the galvanometer of a light-sensitive instrument, such as the Weston photometer, at the other end. The ruby glass filter which transmitted only a narrow band of red (an average wavelength of 6,500 Å) was used because in the region of the band mentioned there was a maximum change in

[6] Acknowledgment is made to Parke Davis Company for their kindness in furnishing the hypophyseal glands.

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light transmission with the changing concentration of blue coloring matter and hence maximum sensitivity. Briefly, the purpose was to measure light intensity in that region of the spectrum where the greatest changes in magnitude occurred, irrespective of the color.[1]

From the data obtained by this method, calibration curves for the salinities ranging from 0 o/oo to 30 o/oo (30 gms of salts per 1,000 ml of water) with 5 o/oo increments were constructed. An artificial sea water suggested by Buch was used, in which NaCl, K_2SO_4 and $MgCl_2$ of the highest purity (almost free from phosphorus) were dissolved in water redistilled in a pyrex still. Such water was practically free from phosphorus. Pure KH_2PO_4 was added to these solutions to make them of concentrations of phosphorus ranging from 0 mg P/m³ to 100 mg P/m³ (100 milligrams of phosphorus per cubic meter of water).

Calibration curves, which deviated only slightly from straight lines, were made by plotting as ordinates the logarithm of the photometer reading on semi-log paper, against the concentration of phosphorus in milligrams per cubic meter as abscissae. By observing the point on the calibration curve corresponding to the photometer reading obtained for a test, the phosphorus content may be read off on the abscissa. Such curves have been found satisfactory, because known amounts of phosphorus added to the test solution have been recovered with small error, indicating that the curve and method are satisfactory and reasonably accurate. Table 1 shows the recovery

TABLE 1

Inorganic phosphorus present in water sample	Inorganic phosphorus added	Total inorganic phosphorus expected	Total inorganic phosphorus recovered
mg P/m³	mg P/m³	mg P/m³	mg P/m³
18	0	18	18
18	10	28	28
18	20	38	40
18	30	48	50
18	80	98	96
9	0	9	9
9	10	19	18
9	20	29	30
9	30	39	39
9	80	89	94

of added phosphorus, in milligrams per cubic meter, from Chesapeake Bay water of an approximate salinity of 15 o/oo (first group of figures) and from the same water diluted one half with redistilled water (second group of figures). Due to the use of the ruby glass filter the sensitivity and accuracy of the method were greatly increased, especially in the lower concentrations of phosphorus, as can be seen from the fact that one milligram of phosphorus corresponds to forty divisions on the photometer scale.

The question of the effect of salts on color production needs consideration, since the samples to be tested are saline solutions. A study of the curve for redistilled water as compared with the curves for artificial sea water shows the former to have a steeper slope than the latter, which means that the salts in the water decrease the amount of color. The slope of the curves for artificial sea water were found to decrease a little for the salinities studied, *i.e.*, from 5 o/oo to 30 o/oo. This shows that for the salinities of 5 o/oo to 30 o/oo the effect of the salts on the color production is small, *i.e.*, there is only a small increasing effect with the increasing salinity in the range tested. In fact, an average curve for salinity from 5 o/oo to 30 o/oo and phosphorus content from 0 to 100 milligrams per cubic meter has given results accurate enough for our purposes. But the salt effect or decrease in color due to the presence of salts is considerable when compared with the color in redistilled water. This is readily seen from the curves (see Fig. 1).

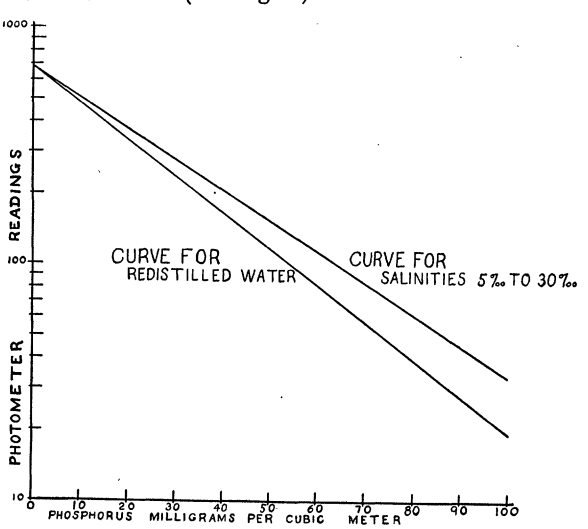

FIG. 1

It was suspected that copper and iron salts have an effect on color production. Accordingly, copper as $CuSO_4$ and iron as $FeSO_4$ and $Fe_2(SO_4)_3$ were added in varying amounts in a series of tests. The amounts of each metal added were such as might be found in estuarine waters, but each series was extended beyond this so as to increase the amount four or five times, thus covering any possible range that might be encountered. The effect of copper and iron on color production was negligible in redistilled water as well as in the various salinities of artificial sea water tested. The interfering effects of silica are prevented in our tests by the high acidity of molybdate sulfuric reagent used in the Denigès method.

While there is a salt effect no corrections need be

[1] In this investigation the authors are pleased to acknowledge the aid given by Professor A. H. Pfund, of the Department of Physics in Johns Hopkins University, in the solution of problems of light absorption.

made for salinity and other interfering substances if calibration curves are used because these interfering factors with one exception, that of arsenic, are taken care of in the preparation of the curves. Arsenic, if present as arsenate, and phosphorus are determined together in the Denigès method, so it is necessary to evaluate the arsenate by suitable means if true phosphorus values are to be obtained.

Farber and Youngberg[2] have shown that with the Denigès[3] method, copper, iron and sulfates do not interfere with the color production in phosphorus determinations if appropriate amounts of reagents are used. Cooper[4] has recognized that there is an interfering effect of the salts present in sea water. Brujewicz[5] gives tables of correction for various salinities, showing that the latter have an effect on color production, and he evaluates a corresponding factor. Kalle,[6] on the other hand, maintains that in the true sense of the word there is no salt error, but that the effect obtained is due rather to the copper present in the sea water. Using the same instrument as Kalle, Robinson and Wirth[7] found a decided decrease in color intensity due to the presence of salts. Our results show that there is decided salt effect from redistilled water to a salinity of 5 o/oo, but that this effect increases only a small amount from this point to 30 o/oo salinity. Also, in agreement with Farber and Youngberg, copper and iron in the magnitudes tested have no effect on color production.

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A METHOD OF FREEING FUNGI FROM BACTERIAL CONTAMINATION[1]

IN work with fungi it is often necessary to obtain cultures which are entirely free of bacterial contamination. This problem presents particular difficulties with such groups as the aquatic Phycomycetes, in which the whole mycelium is immersed in water or culture medium. Several methods have been described to secure bacteria-free material of such forms, but many of them rely on complicated apparatus, difficult technique or long and time-consuming procedure. The following method, requiring a minimum of effort and time, has been used successfully to free a number of water molds from their bacterial contaminants.

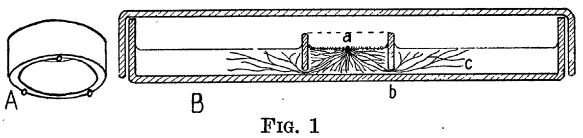

FIG. 1

To one end of a Van Teighem ring are fused three small glass beads, one third to one half mm in diameter, as shown in A of the accompanying diagram. The ring is placed in a Petri dish with the beaded surface resting on the bottom of the dish. Into the Petri dish enough nutrient agar (2 per cent. agar plus some suitable nutrient) is poured to bring its surface well up on the sides of the ring as in B. After the agar has solidified, a bit of inoculum is transferred to the area within the ring (*a*). As growth takes place some of the hyphae extend down into the agar, grow under the ring (b) and into the agar lying beyond it; the contaminating bacteria, however, do not follow the hyphae but are retained at the surface of the semi-solid within the ring. Cubes of agar containing numerous hyphal tips from this outlying portion of the mycelium (c) are therefore bacteria free; from these it is simple to start fresh cultures perfectly free from contamination.

This method has been used so far only for the purpose of freeing water molds from bacteria, but it should prove equally effective in securing pure cultures of any fungus which does not form an aerial mycelium. It is described here in the hope that it may be of use to other students working with such forms.

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