SCIENTIFIC APPARATUS AND LABORATORY METHODS

CONTROL OF ULTRA-VIOLET RAY LAMPS

INVESTIGATIVE work with ultra-violet ray lamps is handicapped considerably by the difficulty in obtaining equal doses of irradiation during a time unit in serial experiments. Not only will irradiation provided by different lamps of the same make vary widely, but also the effect of one lamp at different times. For accurate experimental results it is therefore necessary to determine the irradiation power of the lamp frequently, if not before each experiment. A simple method of ultra-violet lamp control is employed in the writer's laboratory.

The iodized oil preparations which are in clinical use for roentgenographic contrast effect (in bronchography, etc.) are chemical compounds of vegetable oils and of a high percentage of iodine. The French preparation "Lipiodol" (Lafay) which we use is a compound chiefly of poppy-seed oil and 40 per cent. of iodine. Its content of free iodine seems to be quite constant at .03 mg per cc of Lipiodol. Under the influence of daylight additional iodine is freed slowly from the compound, while under irradiation with ultra-violet light the decomposition of the oil occurs with great swiftness, beginning after 35 seconds of irradiation. Determination of the free iodine content after irradiation over a given period furnishes an excellent indication of the power of the lamp at the moment. The following procedure of irradiation and of titration of free iodine is employed.

For practical purposes it is sufficient to employ one

TITRATION OF IRRADIATED LIPIODOL FOR FREE IODINE

Distance of ultra-violet lamp: 30 cm.

Lipiodol in Stender dish, layer 2½ mm.

Dissolve 2 cc of the irradiated Lipiodol in 10 cc CCl_4 (Carbon tetrachloride).

Add 10 cc of 10 per cent. KI (Potassium Iodide).

Acidify with HCl. Starch as indicator.

Titrate with 1/1000 N Sodium Thiosulphate ($Na_2S_2O_3$)

$$\text{Calculation: } \frac{\text{gms } Na_2S_2O_3}{\text{MW } Na_2S_2O_3} = \frac{X}{\text{MW } I_2}$$

X = gms I_2 freed from Lipiodol

EXAMPLE OF RESULTS

Amount of Lipiodol	Time of irradiation	cc 1/1000 N $Na_2S_2O_3$	mg Iodine per cc of Lipiodol
2 cc	15 sec.	.5	.032
2 "	35 "	.5	.032
2 "	1 min.	.9	.057
2 "	2 "	1.6	.101
2 "	3 "	2.0	.127
2 "	4 "	2.4	.152
2 "	5 "	2.5	.158
2 "	6 "	2.7	.171
2 "	7 "	3.6	.228
2 "	10 "	5.1	.323

irradiation period and, for instance, judge the power of the lamp by the free iodine content in the oil after five minutes' irradiation.

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CELLOPHANE AS A SUBSTITUTE FOR MICA

I AM taking this means of acquainting my fellow workers in the field of mineralogy and petrology with the uses I have made of cellophane. Any one who has attempted to make quarter-wave mica plates or to build up a mica wedge or to make a Bravais Test Plate is well acquainted with the difficulty encountered in obtaining plates of mica of the same thickness. I accidentally discovered that cellophane was doubly refractive and remarkably uniform in thickness. With this material I have made practically every kind of accessory for which mica is usually employed, with very little effort because of the abundance of the material. The usual accessories are rather expensive and schools and colleges can rarely afford to supply all their pupils with them. It would be perfectly possible and I believe would lead to a better understanding of the phenomena revealed if the pupils were taught to make their own accessories using this material.

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SPECIAL ARTICLES

HISTOLOGICAL BASIS OF SEX CHANGES IN THE AMERICAN OYSTER (OSTREA VIRGINICA)

IT has long been known[1] that the European oyster (*O. edulis*) is hermaphroditic, with more or less regularly alternating changes of sex during its lifetime. More recently the writer[2] found that in *O. lurida* on the coast of southern California similar changes of sexual phase occur in rhythmical sequence, following a preliminary male phase. In the latter species the gonads of young animals always contain both ovogonia and spermatogonia, but the female cells are delayed in their development until after the completion of the male phase. Following the discharge of most of the sperm the female phase is assumed, but before ovulation has occurred residual spermatogonia

[1] J. H. Orton, *Jour. Mar. Biol. Ass.*, 14: 967–1045, 1926–27.

[2] W. R. Coe, SCIENCE, 74: 247–249, 1931; *Bull. Scripps Inst. Oceanog., Tech. Ser.*, 3: 119–144, 1932.

which did not participate in spermatogenesis during the first male phase now engage in a rapid proliferation to form the gametes of the second male phase, which usually follows the female phase immediately. After a period of recuperation the animal again functions as a female and the sequence is repeated.

In *O. virginica,* on the other hand, the adults are, with few exceptions, of separate sexes. About a year ago Burkenroad[3] produced evidence to show that on the coast of Louisiana this species is regularly protandric. He concluded that all individuals in that region first become sexually mature as males and that with increase in size some of them change to the opposite sex. The proportion of these young males which will thus reverse their sex was thought to depend in some measure, at least, upon the location of the individual with reference to other and larger individuals. Those which are closely associated with others more frequently retain the male phase than do those which are widely separated, for the proportions of females among isolated individuals was found to be much greater than among those growing in clusters.

Quite recently Alfreda Needler[4] has shown that sex reversal actually occurs in the adult oyster, for one of the three males which survived her experiment was found to be of the opposite sex when examined the following year. The work of Amemiya[5] on the Japanese oyster (*O. gigas*) is thought to indicate that sex in that species is differentiated each winter without regard to the sexuality of the animal during the preceding year.

In order to determine, if possible, the histological explanation for the sexual conditions found in *O. virginica,* serial sections of the gonads of young oysters of approximately known age have been made at frequent intervals during their first two years of life.

The study shows that the early gonads in all individuals examined are histologically similar, containing both ovogonia and spermatogonia, but in widely different proportions. At the age of about four months, or when the shell has reached a length of about 12 to 20 mm, the young ovocytes become conspicuous by their rapid growth and the presence of deeply staining mitochondrial bodies (yolk nuclei). Synapsis and spireme-formation precede the growth stages. In the same follicles, and interspersed among the ovocytes, are much greater numbers of spermatogonia in rapid proliferation, leading to the formation of the primary spermatocytes in which synapsis and spireme-formation likewise occur.

As the temperature of the water becomes lower, toward the end of November in Long Island Sound and on the south shore of Long Island, the growth of the ovocytes is inhibited and the proliferation of spermatogonia ceases. During the winter months there is but little change in the appearance of the gonads, but activity is resumed in early spring unless the nutritive conditions of the individual are retarded.

At this season sexual differentiation occurs in many of the larger individuals. The character of the gonads then changes, either by the continued proliferation of spermatogonia and the formation of spermatocytes, as is more usual, or by the rapid growth of the ovocytes in the small proportion of animals that are to become functional females during their first year. In the former case many of the previously formed ovocytes gradually become pycnotic and eventually disintegrate, although large numbers of indifferent gonia and usually some ovocytes remain as residual cells after spermatogenesis has been completed. In such residual cells lies the prospective potency of a later sex reversal.

A much smaller number of the young oysters, however, during the past season in the areas under investigation, became functional females during their first year. In the gonads of these most of the spermatocytes became pycnotic and eventually disintegrated during the process of ovogenesis, although usually some spermatocytes remained as residual cells.

Not infrequently during this process of sexual differentiation different follicles in the same gonad showed opposite sexual characteristics and in some cases true hermaphroditism continued into the breeding season. The eggs of such individuals appear to develop normally after self-fertilization in spite of the vast excess of sperm with which they are surrounded.

The samples examined indicate that in New Haven harbor less than 3 per cent. of the oysters that become sexually mature during their first year are females, while at West Sayville, Long Island, where they reach a much larger size at the age of one year, the percentage of females is between 20 and 30. The ovaries of these develop directly from the primary intersexual gonad without passing through a true preliminary male phase. The species is thus only partially protandric, and it is conceivable that the more numerous males may include both genetically "true males" and protandric males.

Sex changes in this and possibly in other species[5] of seasonably dioecious oysters are evidently based upon the essential hermaphroditism of the primitive gonads and the retention of residual cells of different sex potencies in the individual at the end of the breeding season. And since, as a rule, only the largest of the oysters develop as females during their first

[3] M. D. Burkenroad, SCIENCE, 74: 71–72, 1931.

[4] Alfreda B. Needler, *Cont. Can. Biol. and Fish.,* 7: 285–294, 1932.

[5] I. Amemiya, *Proc. Imp. Acad. Tokyo,* 5: 284–286, 1929.

year, with a much larger proportion of females where the nutritive conditions are better or extend over a longer period, as indicated by the size attained during the first year, it is assumed that the sexuality of the animal is closely correlated with its nutrition. The observation that males are more frequent among closely clustered groups than among isolated individuals may likewise be indicative merely of different nutritive conditions at the critical period of sex differentiation, and is not considered proof that the early male phase is retained because of the hormonic influence of a neighboring individual.

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AUTOLYZED LIVER THERAPY IN PERNICIOUS ANEMIA

FRESH beef liver from slaughter house was ground and $\frac{1}{50}$ Normal HCl added in the proportion of 2,000 gms minced liver to 5,000 cc $\frac{1}{50}$ Normal HCl. Chloroform was added as a preservative. This mixture was placed in an incubator at 37° C. and allowed to undergo autolysis for an average period of 10 days. Portions of this autolyzed liver preparation after partial concentration under reduced pressure were fed by mouth to 3 otherwise untreated cases of pernicious anemia, showing classical clinical features with symptoms indicating cord changes, typical blood picture and achlorhydria. Reticulocyte responses characteristic of the treatment of pernicious anemia with potent material varying from 10 to 16 per cent. followed the oral ingestion of the equivalent of 500, 750 and 800 grams of liver, respectively, in the three cases studied.

The following figures summarize the finding in Case II of the reticulocyte response to autolyzed liver.

Date	Reticulocytes per cent.	Grams autolyzed liver fed
June 3, 1932	1.0	60
4	.5	150
5	2.0	300
6	1.0	300
7	1.5	
8	3.0	
9	7.0	
10	13.0	
11	9.0	

Riddle and Sturgis[1] report that the equivalent of approximately 3,000 grams of liver when fed in single massive doses by mouth in the form of Lilly's Extract No. 343 was required to induce a maximal reticulocyte response.

A comparison of the amounts of liver reported in the literature necessary to invoke a maximal reticulocyte response with the amounts of liver used above suggest that autolysis may increase the potency of the liver preparation.

This is of interest in relation to the recently reported findings of the influence of gastric juice and extracts of stomach on beef and liver.

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[1] Matthew C. Riddle and Cyrus C. Sturgis, *Am. J. Med. Science*, Vol. 180, page 1, July, 1930.

A NEW METHOD FOR THE DEMONSTRATION OF ANTIGEN-ANTIBODY COMBINATION

THE method for demonstration of antigen-antibody interaction is as follows:

A rabbit sensitized to some animal protein (blood serum, egg albumin, etc.), receives an intradermal injection of 0.25 cc of undiluted bacterial filtrate of ascertained skin-preparatory potency.[1] Twenty-four hours later the rabbit is injected intravenously with the same animal protein. From four to five hours later there appears severe hemorrhagic necrosis at the prepared skin site. The lesion is characteristic of the phenomenon of local skin reactivity to bacterial filtrates[2].

Inasmuch as the necessary state of sensitization can be obtained by a single injection of a protein and one-week incubation period is sufficient, the method offers the advantages of speed and simplicity. The readings are reliable and clear-cut, since the incidence of positive results is high (*i.e.*, with some proteins about 85 per cent. of rabbits tested after a single sensitization) and since the severe hemorrhagic necrosis makes the reaction unmistakable. The test is highly sensitive (*i.e.*, dilution 1:10.000 of human serum elicited the necessary sensitization) and strictly specific unless repeated sensitizing injections are made. Because anaphylactic shock in rabbits is difficult to elicit, the test injection of animal protein has no lethal effect on these animals.

It is also possible to elicit a severe reaction in prepared skin sites of non-sensitized rabbits receiving separate (one half hour apart) intravenous injections of antigen and antibody (*i.e.*, passive transfer).

The test is to be clearly differentiated from the

[1] G. Shwartzman, Proc. Soc. Exp. Biol. and Med., 26, 843, 1929; *J. Inf. Dis.*, 44, 232, 1929.

[2] G. Shwartzman, *J. Exp. Med.*, 48, 247, 1928.