cludes, that the CN resistant respiration is not a residual part of the normal respiration, but arises under the influence of cyanide. This view is supported by the lower temperature coefficient of the cyanide stable respiration.[7]

RQ of the developing sea urchin egg is known to be about 0.90 (for literature see[8]). Using a modification of the method of Dickens and Simer[9] my co-worker Öhman[10] recently determined RQ of the 1–2 hour stage as 0.73 ± 0.01 and of the 7–8 hour stage as 0.85 ± 0.01. With the 3 manometer method of Warburg I find an RQ of the cyanide resistant respiration of 1.22 ± 0.01 and an almost equal value in both the above mentioned stages, which means a considerably higher value than for the normal respiration. This excludes that an oxidation of cyanide to cyanate could be an important factor of the cyanide stable respiration as suggested for yeast by Pett.[11]

Using a strain of bakers yeast from Rotebro without substrate I have found HCN (0.001 m) to cause an increase of RQ. With rising oxygen pressure the O_2 consumption as well as the CO_2 production rises, the latter more than the former. This proves that CO_2 is not formed by fermentation. An analysis of HCN and NH_3 reveals that a complete oxidation of HCN does not form any considerable part of the cyanide stable respiration. This will probably be the case for sea urchin eggs also. The high RQ of the cyanide stable respiration of the sea urchin egg suggests either a complete oxidation of some substrate with this RQ or an oxidative decarboxilation taking place. The first substrates of this kind to be considered are glyceric acid and pyruvic acid (RQ = 1.20). Both these compounds, the latter in the form of cyanhydrin, increase the cyanide resistant respiration of the sea urchin egg as long as it has not reached its maximal value. Once this rate is reached there is no effect at all.

The inhibition of the respiration in the unfertilized sea urchin egg by cyanide is known to be rather small.[12, 13] In view of the above reported facts, it seems possible that a larger inhibition in the unfertilized egg may be masked by a cyanide resistant respiration. For further investigation the cyanide resistant respiration of the fertilized and unfertilized egg (0.001 Mol KCN) is measured at different oxygen pressures, ranging from 3 to 100 per cent. The function of the dependence on the oxygen pressure is the same in both cases. The CN resistant respiration does not increase at the same rate as the normal respiration at the fertilization and is in the fertilized egg proportionally much smaller than in the unfertilized. It is thus impossible to determine the real inhibition caused by cyanide in the unfertilized sea urchin egg. In 3 per cent. O_2 the inhibition was at least 70 per cent. Under the same O_2 pressure the normal respiration decreased by 3 per cent. A full description of the experiments will appear in *Archiv för Kemi och Mineralogi,* Stockholm.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

SPIRIT BLUE AGAR: A MEDIUM FOR THE DETECTION OF LIPOLYTIC MICROORGANISMS

THE detection of lipolytic microorganisms is important in the study of certain types of food spoilage, in taxonomic bacteriology and in other phases of microbiology. In a recent comparative study,[1] it was noted that each of the media which is used conventionally for the detection of lipolytic organisms falls short in one respect or another. Those media in which a dye is employed as the indicator of lipolysis are often quite toxic for certain types of microorganisms; *e.g.,* Turner's Nile blue sulfate technique inhibits the growth of micrococci.[1, 2] A sensitive differential medium which could be prepared without difficulty, which would not harm appreciably the growth of the organisms and which would permit indisputable detection of fat-splitting microorganisms would be of value in the study of lipolysis.

A medium of the following composition meets these requirements:

Agar	30.0 gm
Tryptone	10.0 "
Yeast extract	5.0 "
*20 per cent. cottonseed oil emulsion	25.0 ml
0.3 per cent. alcoholic solution of Spirit Blue (National Aniline)	50.0 "
Distilled water to make	1000.0 "

* Prepared by grinding thoroughly in a mortar or colloidal mill: 100.0 ml of *fresh* cottonseed oil, 10.0 gm finely powdered gum arabic and 400.0 ml of warm distilled water. Thorough grinding will result in a smooth, permanent emulsion in which most of the fat globules are less than 10 micra in diameter. Certain samples of cottonseed oil, because of high acidity, are unsatisfactory for use in spirit blue agar; Wesson oil is entirely satisfactory for this purpose.

[7] I. Korr, *op. cit.*
[8] *Archiv f. Zool.,* 32 A, N:o 15.
[9] *Biol. Jour.,* 905, 1930.
[10] *Archiv f. Zool., op. cit.*
[11] *Biol. Jour.,* 30: 1438, 1936.
[1] H. F. Long and B. W. Hammer, *Iowa State College Journal of Science,* 11: 343–351, 1937.
[2] R. H. Turner, *Jour. Inf. Dis.,* 44: 126–133, 1929.
[12] J. Runnström, *op. cit.*
[13] I. Korr, *op. cit.*

The agar, tryptone and yeast extract are dissolved in approximately 900 ml of distilled water by autoclaving for several minutes. After *complete* solution of these components, the cottonseed oil emulsion and the previously filtered alcoholic spirit blue solution are added. The mixture is made up to one liter with distilled water and mixed thoroughly. The medium is sterilized by autoclaving for 15 minutes at 15 pounds (121° C.). Either pour-plates or streak-plates may be used. The completed medium should be stored in a refrigerator to minimize oxidative deterioration; refrigerated sterile plates of spirit blue agar will keep for more than two months.

When prepared from fresh cottonseed oil of a low acid number, sterile plates of spirit blue agar are pale lavender in color and of firm consistency. Colonies of lipolytic organisms are recognized by the development of a permanent deep-blue color beneath and surrounding the colony. No comparable change in color has been detected around colonies of any non-lipolytic organism examined.

The growth characteristics and lipolytic activity of more than 200 species of bacteria, yeasts and molds were examined by means of spirit blue agar. In no instance was there observed an inhibition of growth or of lipolysis which might be attributed solely to the dye; particularly significant is the excellent growth of all the Micrococcaceae which were examined. By use of this medium, it is possible to get "total" counts on dairy products, air and sewage which compare favorably with those obtained by the standard quantitative methods for the examination of these products, and, at the same time, the numbers of lipolytic organisms may be determined.

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AN IMPROVED METHOD OF APPLYING COLORED PENCILS

THE procedure described below is so simple and useful that it is difficult to believe that it is not already in widespread use, yet in the course of a varied experience in America and Europe the writer has not previously encountered it. Locally, at least, it is the invention of our Spanish draftsman:

To secure a uniform and smooth distribution of the color applied by any good grade of colored pencil, it is only necessary to rub the surface to which the pencil coloring has been roughly applied, with a bit of cloth soaked in gasoline. The cloth may be wrapped over the end of a toothpick or the blunt end of a penholder for finer work, and simply bunched into a soft mass for larger areas. The procedure is thus identical with the dry rubbing usually employed to obtain a smooth distribution of crayon coloring. The results obtained by using gasoline compare favorably with a good grade of water-coloring. Much less skill is required to obtain good results, and the work can be done much more rapidly than with paints. Pencil colors so treated are completely fixed, and will not rub off or smudge.

Through the use of gasoline any two pencil colorings may be mixed to obtain a third. Thus, to make a yellow-green, apply first a rough base of yellow, and over it an equally rough surface of green. Upon rubbing with a bit of gasoline-soaked cloth, the two will blend smoothly to produce a yellow-green. Where the colors are of equal value in such mixtures, the color applied uppermost will predominate.

The above technique should be particularly useful to cartographers and others who wish to apply rapid crayon coloring over relatively large surfaces. In the absence of gasoline, turpentine will serve the same purpose, but the oily components of this substance have an adverse effect on lighter grades of paper.

BRANDON H. GROVE

VACUUM OIL COMPANY,
MADRID, SPAIN

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