cc fluid, powdered egg white. This is added just slowly enough so that no flocculation occurs. The fluid is then put in 15 cc centrifuge tubes to the amount of 9 cc per tube. The Opalinas are introduced by a capillary pipette after having been removed from the cloaca of the infected frog and having been washed once in either Ringer's or Pütter's fluid, without the egg white. This last procedure tends to reduce the amount of extraneous matter. About .1 cc of fluid containing a heavy concentration of Opalinas is introduced into each tube. These tubes are then corked with one-hole rubber stoppers. As a final seal, the holes of the stoppers are closed with small glass plugs. This arrangement permits the escape of the air that would otherwise be confined under pressure in the tubes. The presence of this pressure was found by Konsuloff to kill the cultures. All the glassware and corks were sterile before using.

In order to observe the animals without removing them from the tubes and thereby introducing air and bacteria which would lessen the length of the period of cultivation, a binocular microscope was set up horizontally and the tubes held singly at the correct focal distance.

We hope that this technic in the cultivation of Opalinas will be of some help to others working in this field.

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## A SIMPLE METHOD OF OBTAINING SINGLE-SPORE CULTURES

WHILE working with species of Fusarium the writer developed a method of obtaining single-spore cultures that seems simpler and more rapid in operation than methods previously described. A piece of soft glass tubing is heated over a Bunsen flame and drawn out to an inside diameter slightly greater than that of the spores to be isolated. Care in the amount of heat to be applied and a little practice in manipulation is necessary to obtain suitable diameters. The prepared capillaries are broken up into lengths of about three cm and filled by means of capillary attraction from a spore suspension made in warm nutrient agar. After a few trials a spore dilution is easily made that will give from one to four spores to the tube.

The filled tubes are then examined under the microscope and broken up according to the number and position of the spores observed. The resultant pieces are again examined to make sure that each contains one spore only, then picked up with forceps, immersed in alcohol to sterilize the outside and placed in the desired medium. By using a solid rather than a liquid medium the filled tubes may be broken without disturbing the contents and sterilized without injury to the spores.

Should it be desirable to obtain cultures from a single hypha, then the piece of glass tube with the spore is pushed to the bottom of the medium in a Petri dish where germination may be followed under the microscope; as soon as the germination hypha has grown beyond the end of the tube it is cut off and the glass removed. A single hypha is very easily obtained in this manner because of the macroscopic size of the glass tube.

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# SPECIAL ARTICLES

## DIET AND BODY FAT

IN a study[1,2] of the influence of diet upon the quality of fat produced in the animal body we found when rats were fed diets containing dried skimmed milk and either peanut oil or soybean oil or corn oil, these dietary oils furnishing about 60 per cent. of the total food calories in each case, the fat or rather oil yielded by the rat in each case was quite similar in iodine number value to that of the food oil. On the other hand, when a diet containing dried skimmed milk and starch (the latter being substituted equicalorically for the oil ingredient of the above diets) was fed, a so-called "hard" fat was obtained. Under all experimental conditions, cod liver oil and yeast were added to the ration as sources of vitamins.

Furthermore, we found it possible to convert the "soft" body fat into a "hard" body fat by changing the oily diet to the carbohydrate-rich diet, provided the change of food took place when the rats were of adolescent age (140–150 gm.) and the feeding of the "hardening" diet was continued over a comparatively long period. For example, the "soft" body fat of 140 gm. rats produced on a soybean oil diet was completely "hardened" on the carbohydrate-rich diet when the latter was fed until rats attained the weight of about 250 gm.

The question naturally arose: What would be the effect of fat depletion through selective starvation on the subsequent rate of "hardening" of the body fat?

In seeking an answer we subjected rats, grown to various weight levels on the oil-containing diets, to a starvation process before feeding the "hardening" diet. We then compared the fat obtained from other

[1] Anderson, W. E., and Mendel, L. B., "A Technique for the Study of Fat Production in Animals," Proc. Soc. Exp. Biol. and Med., 1923–24 (21), 436.

[2] Anderson, W. E., "The Influence of Diet on Fat Production in the Animal Body," Proc. Am. Soc. Biol. Chem., *J. Biol. Chem.*, 1925 (63), XLVI.