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.

A. RANGER TYLER

DEPARTMENT OF ZOOLOGY,
RUTGERS UNIVERSITY

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.

H. N. HANSEN

PLANT PATHOLOGY LABORATORY,
UNIVERSITY OF CALIFORNIA

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.

rats raised under exactly the same dietary conditions but not subjected to the fat depletion treatment through starvation.

TABLE I

Rats 1456, 1463, 1464, 1448, 1454 and 1462 (all males) raised to 250 gm. (± 3.0 gm.) on D.S.M. (dried skimmed milk) and soybean oil.

Rat
1456 starved from 248 gm. to 184 gm. (25.8 per cent. loss)
1463 starved from 248 gm. to 182 gm. (26.6 per cent. loss)
1464 starved from 247 gm. to 185 gm. (25.1 per cent. loss)

Rat		Killing weight
1456	fed D.S.M. and starch from 184 gm. to	275 gm.
1463	fed D.S.M. and starch from 182 gm. to	280 gm.
1464	fed D.S.M. and starch from 185 gm. to	272 gm.

Rat		Killing weight
1448	fed D.S.M. and starch from 251 gm. to	280 gm.
1454	fed D.S.M. and starch from 250 gm. to	288 gm.
1462	fed D.S.M. and starch from 249 gm. to	268 gm.

Rat	Iodine number (Hanus)	D.S.M. and starch diet consumed gm.	in days
1456	71.4	453	27
1463	76.0	533	35
1464	71.5	435	27
1448	115.5	442	31
1454	108.5	355	26
1462	109.9	406	28

(123.0 = iodine number of fat produced by rats fed D.S.M. and soybean oil.
60.0 = iodine number of fat produced by rats fed D.S.M. and starch).

In Table I will be found data obtained with six male rats, all of which were raised to a body weight of 250 gm. (± 3.0 gm.) on a diet containing liberal inclusion of soybean oil. During the period of (partial) starvation, to which rats 1456, 1463 and 1464 were subjected, the soybean oil diet was entirely withdrawn, but the vitamin-bearing materials were supplied daily to prevent body depletion thereof.

It will be noted that the fat produced by the starved group of rats is distinctly "harder"—using the iodine number value[3] as a measure of "hardness"—than the fat yielded by the non-starved group. For ready comparison the iodine number values of fat produced by rats fed the soybean oil diet and by other rats fed the diet rich in carbohydrate are added. The amount of the dried skimmed milk and starch diet—"hardening" food—consumed by both the starved and non-starved groups, and the number of days during which it was ingested, are also indicated.

TABLE II

Rats 1488, 1479, 1487, 1478, 1490 and 1492 (all males) raised to 175 gm. (± 3.0 gm.) on D.S.M. (dried skimmed milk) and peanut oil.

Rat
1488 starved from 172 gm. to 126 gm. (26.7 per cent. loss)
1479 starved from 175 gm. to 128 gm. (26.9 per cent. loss)
1487 starved from 175 gm. to 134 gm. (23.4 per cent. loss)

Rat		Killing weight
1488	fed D.S.M. and starch from 126 gm. to	228 gm.
1479	fed D.S.M. and starch from 128 gm. to	223 gm.
1487	fed D.S.M. and starch from 134 gm. to	221 gm.

Rat		Killing weight
1478	fed D.S.M. and starch from 177 gm. to	225 gm.
1490	fed D.S.M. and starch from 173 gm. to	224 gm.
1492	fed D.S.M. and starch from 175 gm. to	225 gm.

Rat	Iodine number (Hanus)	D.S.M. and starch diet consumed gm.	in days
1488	61.9	301	20
1479	63.5	339	21
1487	68.0	365	24
1478	78.3	372	21
1490	75.0	345	24
1492	74.3	314	24

(94.0 = iodine number of fat produced by rats fed D.S.M. and peanut oil.)
60.0 = iodine number of fat produced by rats fed D.S.M. and starch.)

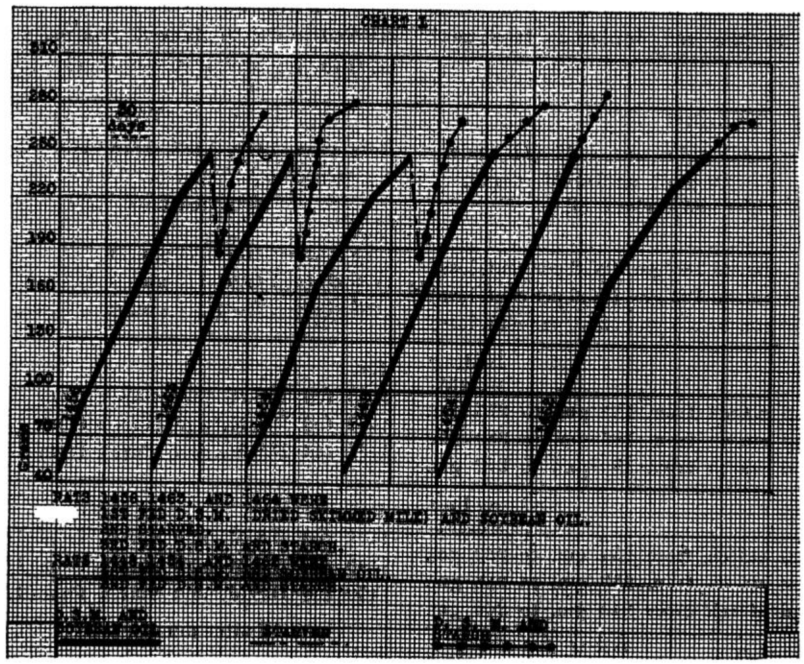
In Table II will be found similar data obtained with six male rats, all of which were raised to 175 gm. (± 3.0 gm.) on a diet containing peanut oil. The method of conducting and the details of this experiment were precisely the same as those referred to in Table I.

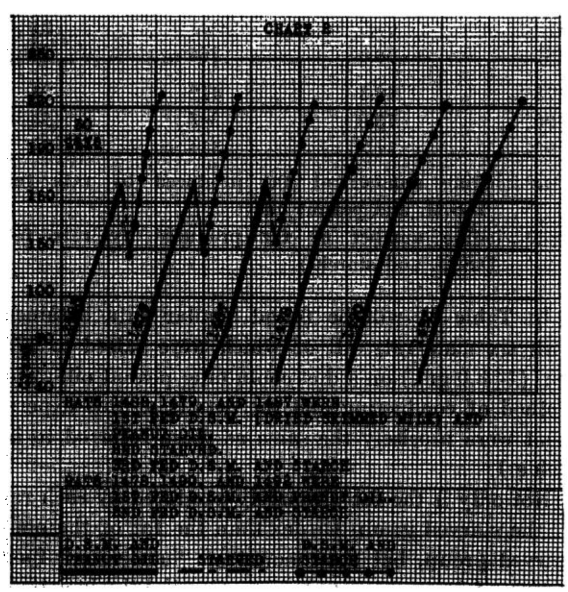
Not only is the fat ultimately produced by each rat of the starved group harder than the fat of the non-starved group, but the amount of "hardening" food consumed by the starved group is slightly less than that consumed by the non-starved lot. This is a striking example—and we have noted other similar instances in our present studies—of the food economy, measured in calories, in the starvation-recovery process.

By reference to the accompanying growth curve charts I and II one can readily visualize the very rapid growth experienced during recovery by all animals of both starved groups.

The results referred to are typical of others which we could furnish. We have performed similar ex-

[3] Ellis, N. R., and Isbell, H. S., *J. Biol. Chem.*, 1926 (69), 237, in their "Soft Pork Studies" state "the iodine and refractive index values were an excellent measure of firmness of the adipose tissue."





periments in which rats have been raised to various weight levels on diets containing corn oil in addition to those containing soybean and peanut oils.

Experiments are in progress in which rats are being subjected to a longer starvation process, involving over 30 per cent. loss in weight. Upon completion of these studies the results will be published *in extenso*.

Summary

Rats first fed soybean oil and peanut oil diets, then subjected to the process of fat depletion through selective starvation, involving 23 to 27 per cent. loss in body weight, before being fed a "hardening" diet, yielded "harder" fats—fats of lower iodine number values—than the fats of rats which were not starved before being fed the carbohydrate-rich diet. In other words, through the process of starvation, the "soft" oily fat produced on diets containing soybean[1] or peanut oils is very largely removed, thereby permitting the deposit of a "hard" fat. To obtain a fat of equal "hardness" from rats which were not subjected to the starvation treatment would have required a much longer period of feeding of the diet rich in starch than was found necessary with rats after first being starved.

The growth of recovery made by the rats of the starved lots was made on a low food intake. With the starved rats first fed peanut oil, the food intake of the carbohydrate-rich diet was less than with the non-starved group.

The possible application of these findings to practical animal husbandry is obvious.

Lafayette B. Mendel,
William E. Anderson*

Laboratory of Physiological Chemistry,
Yale University

[1] The Proctor and Gamble Company, Ivorydale, Ohio, kindly furnished the soybean oil used in these particular experiments.

* Fellow of the Institute of American Meat Packers.