TABLE I. Effect of Corn Oil and Hydrogenated Coconut Oil on Body Weight, Serum Cholesterol, and Liver Cholesterol.

| Subject | Diet | Body wt Initial, lb | Body wt Final, lb | Body wt % change | Serum cholesterol Initial, mg % | Serum cholesterol Final, mg % | Serum cholesterol % change | Liver cholesterol Initial mg/100 g wet tissue | Liver cholesterol Final mg/100 g wet tissue | Liver cholesterol % change |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Hydrog. | 179½ | 180½ | + .6 | 261 | 301 | +15 | 281 | 282 | 0 |
| 2 | coconut | 216¼ | 213½ | −1.3 | 305 | 329 | + 8 | 343 | 315 | − 8 |
| 3 | oil | 170½ | 167½ | −1.8 | 316 | 316 | 0 | 1459 | 1218 | −17 |
| 4 | | 181½ | 180 | − .8 | 267 | 278 | + 4 | 428 | 559 | +31 |
| 5 | | 143½ | 142½ | − .7 | 280 | 264 | − 6 | 353 | 534 | +51 |
| 6 | | 172 | 175 | +1.7 | 265 | 273 | + 3 | 319 | 307 | − 4 |
| | Avg | | | − .4 | | | + 4* | | | + 9* |
| 7 | Corn oil | 219¾ | 215 | −2.2 | 279 | 260 | − 7 | 460 | 310 | −33 |
| 8 | | 172½ | 171 | − .9 | 312 | 266 | −18 | 910 | 545 | −40 |
| 9 | | 163¾ | 161 | −1.7 | 294 | 272 | − 7 | 331 | 349 | + 5 |
| 10 | | 201¼ | 204 | +1.4 | 272 | 245 | −10 | 558 | 382 | −32 |
| 11 | | 204¼ | 204 | − .1 | 325 | 298 | − 8 | 531 | 410 | −23 |
| 12 | | 183¼ | 183½ | + .1 | 266 | 251 | − 6 | ‡ | 763 | ‡ |
| | Avg | | | − .6 | | | − 9† | | | −25† |

* Change from initial value not statistically significant.
† " " " " probably significant ($p < 0.05$).
‡ Although cholesterol was present, this specimen contained no detectable protein by the method used. It is assumed that the biopsy represented fat, rather than liver parenchyma.

concentration fell an average of 9% in the men fed corn oil, but did not change significantly in the controls. Liver cholesterol concentration, as measured by liver biopsy, fell an average of 25% in the men fed corn oil. No consistent effect was observed in the controls. It is concluded that the fall in serum cholesterol produced by corn oil feeding in man is probably not due to a shift of cholesterol from the blood to the liver.

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## A Modified Plastic Petri Dish for Cell and Tissue Cultures.* (26480)

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For many years the 60 mm glass Petri dish has served as a standard culture vessel for maintenance of a variety of cell strains and tissue explants *in vitro*. Monolayer cultures have been maintained on the inside bottom of the Petri dish overlaid by a prescribed volume of liquid medium whose pH is maintained by the humidified 5% $CO_2$ environment of the incubator in which the culture dishes are placed. Observations of the growing cultures require an inverted microscope or replacement of the dish top with a device enabling one to bring the objective close enough so that the cells can be viewed with an ordinary microscope. Both sytems leave

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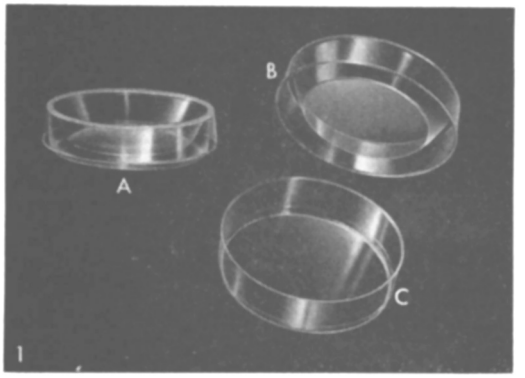


FIG. 1. A. Complete dish, assembled. B. Recessed top. C. Dish bottom.

much to be desired regarding the cytological resolution obtained. The culture dish herein described has many features which tend to overcome some of the problems encountered in growing monolayer cultures in conventional dishes.

The culture dish‡ is made of a pre-sterilized polystyrene plastic. The lid has a sharply recessed area 40 mm in diameter (Fig. 1, B). The sides of the bottom (Fig. 1, C) of the dish are tapered to facilitate their stacking or filing for maintaining permanent records of experiments. The optical working distance from top of the lid to inside bottom of the dish (cell surface) is 2.6 mm. This enables one to study individual cells of the monolayer by using an ordinary light microscope or phase microscope (Fig. 2). When making observations with an inverted microscope (Fig. 3), the recessed lid permits the phase condenser to be focused on the cell surface, thereby producing much better definition of cell structure at higher magnifications. All of these optical procedures do not increase the chance of contamination since all are done with the recessed lid in place.

Despite the presence of the recessed lid, there is ample space between the lid and the bottom to allow for maintenance of the proper pH of the medium by the $CO_2$ environment of the incubator. Approximately 4 ml of medium will flood the bottom of the dish and eliminate the air interface between lid and medium. The maximum working volume of medium is approximately 7 ml.

This dish has been utilized in conjunction with time-lapse cinephotomicrographic technics to record the process of trypsinization in various cell strains and tissues (Fig. 3). Additional experiments involving the applicability of this dish to long term time-lapse cinephotomicrographic experiments are now in progress.

Many histochemical procedures can be carried out completely within this dish. Where the experimental procedure requires an air tight environment, the dish can be sealed by rimming the bottom of the dish

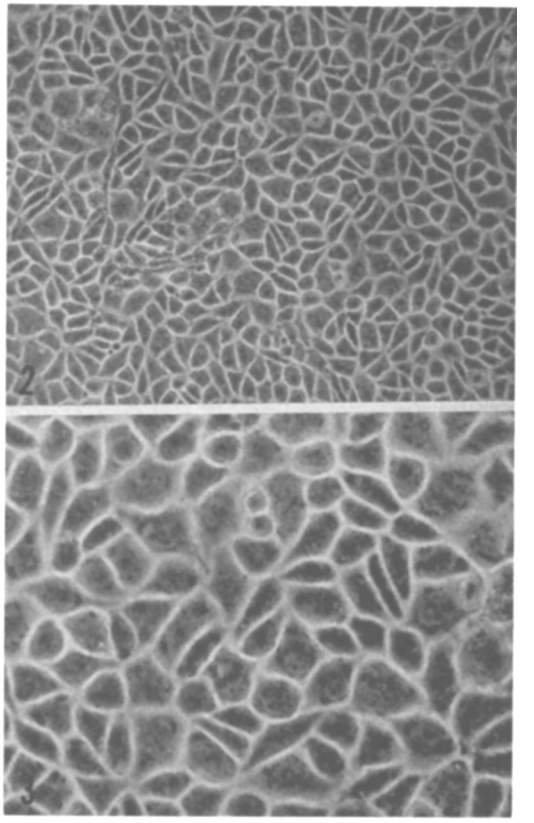


FIG. 2. Photomicrograph of a monolayer culture of L-929 cell strain growing on inner surface of plastic petri dish bottom. The field was photographed on 16 mm Gaevapan movie film from above, through the recessed top, utilizing a 10× A.O. Dark M. phase objective.

FIG. 3. Photomicrograph of a monolayer culture of L-929 cell strain growing on inner surface of plastic petri dish bottom. The field was photographed on 16 mm Gaevapan movie film with the aid of a Zeiss Plankton microscope and a 40× Zeiss phase contrast objective.

‡ Currently available from Falcon Plastics, Los Angeles, Calif.

with silicone or a nontoxic plastic cement.

The design of this dish should aid the investigator in obtaining better cytological detail of monolayer cultures without increasing the danger of contamination and should be especially useful in laboratories where frequent screening is required of large numbers of plates as well as in conducting student laboratory experiments where the supply of inverted microscopes is inadequate.

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## Effect of Glutamate and Glycine on Cock Sperm Metabolism. (26481)

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During investigations on the effect of chloride on cock sperm metabolism it was found that replacement of chloride by glutamate in a Tyrode diluent resulted in a linear depression of the rate of fructolysis and of respiration rate(1). Replacement of chloride by phosphate did not result in a significant change in rate of metabolism. The present paper reports results obtained in a further investigation of these phenomena.

*Materials and methods.* The methods used to obtain semen, to measure respiration rate and utilization of fructolysis have been previously described(1). In all experiments a 1:10 dilution rate was used. All experiments were designed as completely randomized blocks and analyzed according to the methods outlined by Snedecor(2). Diluents were made isotonic to seminal plasma ($\Delta = 0.62°C$). Freezing points were determined with the aid of a Fiske milkcryoscope. Compositions of the basic diluents employed are given in Table I. A designation of 25% "phosphate" means that 3 parts of "glutamate" diluent were mixed with 1 part of "complete phosphate" diluent. All diluents contained 100 mg dihydro streptomyocin and 10 mg tetracycline hydrochloride per 100 ml(3). To determine the catabolism of glutamate 6 $\mu$c of glutamate 1-$C^{14}$ were added per Warburg flask; a similar procedure was used in the case of glycine. The $C^{14}O_2$ was trapped in KOH and either converted to $BaC^{14}O_3$ and the radioactivity of $BaC^{14}O_3$ determined, or the KOH and $KHC^{14}O_3$ were plated directly on in a small metal cup and radioactivity measured on the dried material. Radioactivity was measured with a thin mica window Geiger-Müller counter.

*Experiments and results.* The effect of replacement of chloride by phosphate or glutamate was investigated in an experiment in which the 3 diluents were used simultaneously. The results are presented in Table II. Analysis of variance revealed: 1) Respiration rates of Tyrode-diluted and phosphate-diluted fowl semen are higher ($P<0.001$) than that of glutamate-diluted semen; 2) Diluents had no significant ($P>0.20$) effect on rate of fructolysis. 3) The interaction be-

TABLE I. Composition of Diluents Used in Fowl Semen Experiments.

| Isotonic solution | g/100 ml | "Complete phosphate" | Tyrode | "Glutamate" |
|---|---|---|---|---|
| | | ml | ml | ml |
| $CaCl_2 \cdot 2H_2O$ | 2.04 | 1.00 | 1.00 | 1.00 |
| $MgCl_2 \cdot 6H_2O$ | 2.48 | .40 | .40 | .40 |
| KCl | 1.25 | 1.60 | 1.60 | 1.60 |
| Na H $CO_3$ | 1.53 | 6.50 | 6.50 | 6.50 |
| Fructose | 6.00 | 4.00 | 4.00 | 4.00 |
| Na monoglutamate | 3.21 | — | — | 86.50 |
| Phosphate buffer* | — | 86.50 | — | — |
| Na Cl | .97 | — | 86.50 | — |

* 16.34 g $Na_2HPO_4$ plus 5.16 g $NaH_2PO_4$ in 1000 cc water.