ability of reduced glutathione or of free SH groups in enzymes is considered as a possible mechanism controlling B cell insulin output.

1. Lazarus, S. S., *Fed. Proc.*, 1959, v18, 488.
2. ———, PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 819.
3. ———, *ibid.*, in press.
4. Tobie, J. E., *J. Histochem. & Cytochem.*, 1958, v6, 271.
5. Lazarus, S. S., *A. M. A. Arch. Path.*, 1958, v66, 767.
6. Hess, R., Scarpelli, D. G., Pearse, A. G. E., *J. Biophys. Biochem. Cytol.*, 1958, v4, 753.
7. Scarpelli, D. G., Hess, R., Pearse, A. G. E., *ibid.*, 1958, v4, 747.
8. Lazarow, A., in *Experimental Diabetes*, ed. by Delapresnaye, J. F., and Smith, H. G., C. C Thomas, Springfield, Ill., 1954, p49.
9. Kyrle, J., *Arch. f. mikr. Anat.*, 1908, v72, 141, cited by Cameron, G. R., Pathology of the Cell, Oliver and Boyd, Edin. 1952.
10. Bargman, W., in von Mollendorf, W., *Handbuch der Mikroskopischen anatomie der Menschen*. vVI, 2, Julius Springer, Berlin.
11. Lazarus, S. S., Volk, B. W., *A.M.A. Arch. Path.*, 1959, v67, 456.
12. Nachlas, M. M., Walker, D. G., Seligman, A. M., *J. Biophys. Biochem. Cytol.*, 1958, v4, 467.
13. ———, *ibid.*, 1958, v4, 29.
14. Cascarno, J., Zweifach, B. W., *ibid.*, 1959, v5, 309.
15. Nachlas, M. M., Tsou, K C., De Souza, E., Cheng, C. S., Seligman, A. M., *J. Histochem. and Cytochem.*, 1957, v5, 420.
16. Hogeboom, G. H., Claude, A., Hotchkiss, R. D., *J. Biol. Chem.*, 1946, v165, 615.
17. Pearse, A. G. E., Scarpelli, D. G., *Nature*, 1958, v181, 702.
18. Pearse, A. G. E., Scarpelli, D. G., Brown, S., *Proc. Biochem. Soc.*, 1957, v68, 18p.
19. Baldwin, E., Dynamic Aspects of Biochemistry, Camb. Univ. Press, 1952, p449.
20. Pearse, A. G. E., *J. Clin. Path.*, 1958, v11, 520.
21. Necheles, T., Buetler, E., *J. Clin. Invest.*, 1959, v38, 788.
22. Dickens, F., *Ann. N. Y. Acad. Sci.*, 1958, v75, 71.
23. Mark, P. A., Johnson, A. B., Hirschberg, E., Bank, J., *ibid.*, 1958, v75, 95
24. Gross, R. T., Mark, P. A., *ibid.*, 1958, v75, 106.
25. Hogeboom, G. H., Scheider, W. C., *J. Biol. Chem.*, 1950, v186, 421.
26. Ernster, L., Navazio, F., *Exp. Cell Res.*, 1956, v11, 483.
27. Utter, M. F., *Ann. Rev. Biochem.*, 1958, v27, 245.
28. Glock, G. E., McLean, P., *Biochem. J.*, 1954, v56, 171.
29. ———, *Biochem. Biophys. Acta*, 1953, v12, 590.
30. Siperstein, M. D., *Diabetes*, 1958, v7, 181.
31. Wilson, J. D., Siperstein, M. D., *J. Clin. Invest.*, 1959, v38, 317.

Received September 16, 1959. P.S.E.B.M., 1959, v102.

Essential Growth Factor in Serum Dialysate for Chick Skeletal Muscle Fibroblasts.* (25287)

MORGAN HARRIS† (Introduced by Richard M. Eakin)
Dept. of Zoology, University of California, Berkeley

Growth requirements for mammalian cells in tissue culture have been investigated extensively by Eagle and his associates(3). A number of established cell lines will proliferate indefinitely as monolayers in the presence of 13 essential amino acids, 8 vitamins, 6 ions, and a suitable carbohydrate source, provided that dialyzed serum is added to the culture medium(5,6). Freshly isolated monkey kidney cells have similar short term requirements although indefinite cultivation on Eagle's medium and dialyzed serum has not been achieved(4). Our comparable experiments on chick cells have shown that monolayer cultures obtained from skeletal muscle fail to grow or survive in dialyzed serum supplemented by Eagle's medium. Subsequent work as detailed below indicates that these cells require one or more additional factors present in serum dialysate but not occurring in Eagle's medium or other synthetic nutrients presently available.

* This investigation supported by Cancer Research Funds of Univ. of California and by grant from U.S.P.H.S.

† With technical assistance of Irene Cady.

Material and methods. Freshly isolated chick skeletal muscle fibroblasts were used. Procedures for routine preparation and management of these cells have been described previously(9,11). Stock monolayer cultures were maintained in 10% undialyzed chicken serum (Colorado Serum Co., Denver), 50% Medium 199(16), and 40% balanced saline (Earle's solution) containing 0.4 mg/ml spleen ribonucleoprotein fraction(11). Cell suspensions were prepared by trypsinization and used to inoculate experimental series in T-15 flasks or 1-oz. prescription bottles. A minimal medium designated CS 199 (10% dialyzed chicken serum, 50% Medium 199, 40% Earle's solution) was employed for these cultures and variously supplemented as described below. Dialyzed serum was prepared at 4°C in standard cellophane casings, using platform shaker and frequent changes of a low-bicarbonate saline (Gey's solution) over a 3 day period. Experimental cultures were maintained at 38°C with a daily 50% fluid change, and terminal determinations of cell number were made with a Coulter electronic cell counter(1,10).

Results. A well defined deficiency syndrome develops if dialyzed serum is substituted for whole serum in monolayer cultures of chick skeletal muscle fibroblasts, despite the presence of a synthetic nutrient (Medium 199) at 50-90% in the supernatant fluid. Cells placed in dialyzed serum were at first similar in appearance to populations in undialyzed medium, and proliferated normally for a day or two. Growth then tapered off in dialyzed CS 199 and ceased after 3 to 4 days. Cells in these cultures rounded up or assumed abnormal shapes, became granular and necrotic, and failed to proliferate if subcultured in the same medium. These changes are illustrated by Fig. 1, which shows typical appearance of skeletal muscle fibroblasts after one subculture in dialyzed CS 199. Control cultures in undialyzed chicken serum grew actively to form a continuous sheet of broad, flat cells, and could be subcultured without significant change in appearance of these cells, or diminution in growth rate. It should be pointed out that extensive growth in undialyzed CS 199 occurred regularly but was not

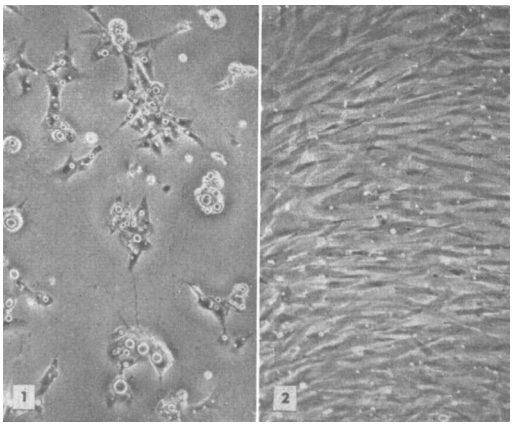

FIG. 1. Typical deficiency state resulting from absence of serum dialysate factor(s). Chick skeletal muscle fibroblasts in 10% dialyzed chicken serum + Medium 199, second passage. Living culture, phase contrast, ×124.

FIG. 2. Prevention of deficiency state by supplementation with proteose-peptone (.1%). Media and cells otherwise similar to culture shown in Fig. 1. Second passage, living culture, phase contrast, ×124.

observed previously using chicken serum from other commercial sources(11). The basis for this difference in sera is under investigation, but preliminary studies show that dialysis of all sera tested blocks growth and prevents maintenance of chick skeletal muscle fibroblasts.

The deficiency syndrome just described was not corrected by substituting a more complex synthetic nutrient, NCTC 109(13) for Medium 199 in the dialyzed assay medium. On the other hand a deficiency state could be completely prevented if serum dialysate was incorporated at an appropriate level in the initial dialyzed CS 199 Medium. These observations suggest that Medium 199 and NCTC 109 as well as the simpler nutrient of Eagle(3,6) are all deficient in one or more factors occurring in serum dialysate, and which are essential for growth and maintenance of chick skeletal muscle fibroblasts in monolayer cultures.

Of various other supplements tested as sources for the missing serum dialysate factor(s), proteose-peptone (Difco) and to a lesser extent tryptose phosphate broth (Difco) proved positive. Addition of proteose-peptone to dialyzed CS 199 gave a clear-cut effect in terms of cell appearance (Fig. 2) as well as

growth stimulation (Fig. 3). At optimal level of 0.1% proteose-peptone the corresponding cell population was indistinguishable in appearance or growth rate from control populations in undialyzed media.

In contrast to the foregoing, negative results were obtained by adding to dialyzed CS 199 supplementary cholesterol, inositol, or vit. B_{12}, all of which have been described as strategic factors for growth of some mammalian strains(6,17,7). Supplementation of dialyzed CS 199 with spleen nucleoprotein fraction(11) also failed to correct the deficiency syndrome. Table I provides a summary of the experiments described above.

Discussion. Failure of 2 synthetic nutrients (Medium 199 and Medium NCTC 109) to correct deficiencies of dialyzed chicken serum indicates that additional requirement of chick skeletal muscle fibroblasts is not to be found

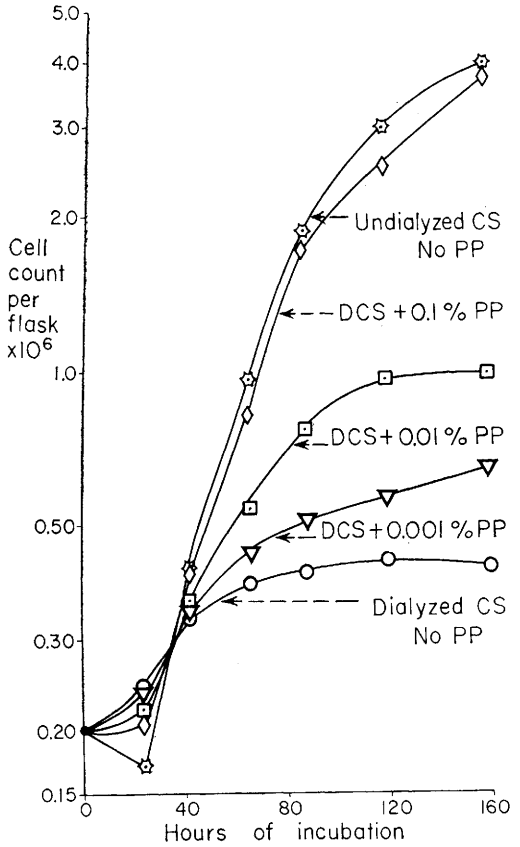

FIG. 3. Growth of skeletal muscle fibroblasts in 10% dialyzed chicken serum (DCS) with 50% Medium 199 and graded levels of proteose-peptone (PP).

TABLE I. Growth of Chick Skeletal Muscle Fibroblasts as Monolayer Cultures in Dialyzed and Undialyzed Media.

Basal medium*	Supplement	Increase in cell count per flask at 5 days, × 10^6†
Undialyzed CS + Medium 199		1.09
Dialyzed CS + Medium 199		.26
Idem	Chicken serum dialysate (1:1), 30% v/v	.97
"	Proteose-peptone, .05%	1.30
"	Tryptose phosphate broth, 10% v/v	1.16
"	Inositol, 10^{-5} M	.30
"	Cholesterol, 1 μg/ml	.29
"	Vit. B_{12}, "	.25
"	Spleen NPF, .4 mg/ml	.38
Dialyzed CS + Medium NCTC 109		.19

* Complete medium: 10% chicken serum, 50% synthetic nutrient, 40% balanced saline (containing supplements where added).
† Avg values. Six flasks/series.

in the more extensive arrays of amino acids, vitamins, and other defined factors present in Medium 199 and NCTC 109 as compared to Eagle's medium. It is significant in this connection that Medium 199 alone, or its subsequent modification, M150(14) does not support progressive growth of fresh explants from chick embryonic heart, or even their survival beyond a limited period(15). The effects of proteose-peptone and tryptose phosphate suggest that the missing factors may include peptides or other split products of protein cleavage. Stimulation of chick cells by proteoses and peptic digests is well documented in the older literature on plasma cultures(2,19), although deficiency states were not described. More recently Gerarde and Jones(8) reported that proteose-peptone, but not an amino acid mixture, increased collagen synthesis for cultures of chick lung in dialyzed media. Peptones or proteose-peptone may also stimulate growth of mammalian cells, although not essential for growth of established strains(12,18).

Summary. Chick skeletal muscle fibroblasts in monolayer cultures grow actively in homologous undialyzed serum supplemented with Medium 199. Dialysis of chicken serum

removes a factor essential for growth and maintenance of these cells. The deficiency syndrome in cultures with dialyzed serum can be corrected by chicken serum dialysate, but not by inositol, cholesterol, vit. B_{12}, spleen nucleoprotein fraction, or by a more complex synthetic nutrient, NCTC 109. Proteose-peptone at 0.1% may be substituted for serum dialysate to restore full activity of whole serum.

1. Brecher, G., Schneiderman, M., Williams, G. Z., *Am. J. Clin. Path.*, 1956, v26, 1439.
2. Carrel, A., Baker, L. E., *J. Exp. Med.*, 1926, v44, 503.
3. Eagle, H., *Science*, 1955, v122, 501.
4. Eagle, H., Freeman, A. E., Levy, M., *J. Exp. Med.*, 1958, v107, 643.
5. Eagle, H., Oyama, V. I., Levy, M., *Arch. Bioch. Biophys.*, 1957, v67, 432.
6. Eagle, H., Oyama, V. I., Levy, M., Freeman, A. E., *J. Biol. Chem.*, 1957, v226, 191.
7. Evans, V. J., Bryant, J. C., McQuilkin, W. T., Fioramonte, M. C., Sanford, K. K., Westfall, B. B., Earle, W. R., *Cancer Res.*, 1956, v16, 87.
8. Gerade, H. W., Jones, M., *J. Biol. Chem.*, 1953, v201, 553.
9. Harris, M., *Growth*, 1957, v21, 149.
10. ———, *Cancer Res.*, 1959,
11. Harris, M., Kutsky, R. J., *ibid.*, 1958, v18, 585.
12. Mayyasi, S. A., Schuurmans, D. M., PROC. SOC. EXP. BIOL. AND MED., 1956, v93, 207.
13. McQuilkin, W. T., Evans, V. M., Earle, W. R., *J. Nat. Canc. Inst.*, 1957, v19, 885.
14. Morgan, J. F., Campbell, E., Morton, H. J., *ibid.*, 1955, v16, 557.
15. Morgan, J. F., Morton, H. J., Campbell, E., Guerin, L. F., *ibid.*, 1956, v16, 1405.
16. Morgan, J. F., Morton, H. J., Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 1.
17. Sato, G., Fisher, H. W., Puck, T. T., *Science*, 1957, v126, 961.
18. Waymouth, C., *J. Nat. Canc. Inst.*, 1956, v17, 315.
19. Willmer, E. N., Kendal, L. P., *J. Exp. Biol.*, 1932, v9, 149.

Received September 16, 1959. P.S.E.B.M., 1959, v102.

Influence of Dose Rate on Radiation Effect on Fertility of Female Mice. (25288)

LIANE BRAUCH RUSSELL, KATHREN F. STELZNER AND W. L. RUSSELL
(Introduced by A. Hollaender)
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.*

Studies of the variables that affect the action of radiation on cell populations may be expected to yield answers concerning both the nature of the radiation effect and the properties of cell population concerned. An understanding of these factors is of special importance in gametogenic cells, for proper interpretation of results of genetic experiments is not possible. It has long been known that, following irradiation with even a relatively low acute dose, female rodents produce only one or a limited number of litters before permanent sterility sets in, a response entirely different from that of males, which regain fertility even after high doses. The basis radiation-induction of permanent sterility in the female is the extremely rapid destruction of immature primordial oocytes, shown for the mouse by Brambell and Parkes(1) and by Murray(2), whose findings were, in general, confirmed recently by Oakberg(3). The careful quantitative studies of Mandl(4) in the rat lead to similar conclusions. (Her paper gives a review of some of the voluminous literature on radiation effects on the ovary.) It has been found that new formation of oocytes does not occur following irradiation(1,3, etc.), supporting the view that oogonia do not persist in the adult. Since the first meiotic division is not completed until shortly before ovulation which, in any case, involves only very few cells at each estrus, practically the entire gametogenic cell population of adult mouse ovary consists of primary oocytes. Diplotene is

* Operated by Union Carbide Nuclear Co. for U.S. Atomic Energy Comm.