15. Crane, R. K. and Krane, S. M., Biochim. Biophys. Acta **20**, 568 (1956).

16. Csaky, T. Z. and Glenn, J. E., J. Appl. Physiol. **12**, 145 (1958).

17. Jervis, E. L. and Levin, R. J., Nature **210**, 391 (1966).

18. Wright, P. H., Am. J. Med. **31**, 892 (1961).

19. Binder, H. J., Spiro, H. M., and Spencer, R. P., Biochim. Biophys Acta **135**, 350 (1967).

20. Mahler, R. J. and Szabo, O., Proc. Soc. Exptl. Biol. Med. **125**, 879 (1967).

Received Sept. 25, 1968. P.S.E.B.M., 1969, Vol. 130.

Effect of Hydrocortisone on Age-Dependent Changes in Lipid Metabolism of Primary Human Amnion Cells *in Vitro** (33691)

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A previous communication described age-dependent changes in lipid metabolism of primary human amnion (PHA) cells *in vitro*. These changes were: (a) increase in incorporation of palmitate-1-^{14}C into cell lipids relative to oxidation of palmitate-1-^{14}C into $^{14}CO_2$, or conveniently referred to as an increase in lipid/CO_2 ratio, and (b) increase in incorporation of cholesterol-26-^{14}C into cell lipids (1). A subsequent publication described the prolongation of postmitotic life span of PHA cell by hydrocortisone (2). We now ask the question whether hydrocortisone would reverse the age-dependent change in lipid metabolism of PHA culture.

Materials and Method. Cell culture. The preparation and maintenance of replicate PHA cell cultures, the determination of cell density and the nutrient medium used in the feeding of the cultures and the procedure for hydrocortisone experiemnts were essentially the same as those previously described (2) except that no mycostatin was added to the medium.

Isotopes. Palmitate-1-^{14}C, oleate-1-^{14}C, and cholesterol-26-^{14}C with specific activity, respectively, of 6.5, 8.7, and 26.8 mCi/mmole were purchased from New England Nuclear Corporation. The fatty acids were added to nutrient medium as sodium salts to final concentration of 0.5 μCi/ml. Cholesterol-26-^{14}C was first dissolved in alcohol and then dispersed in nutrient medium also to 0.5 μCi/ml. The final concentration of alcohol in medium was less than 0.1% which was not toxic to PHA culture.

Incorporation experiment. This is in general similar to that described previously (1,2). Briefly, 11 days after explantation the amnion cells from one placenta were harvested and distributed to culture tubes each receiving the same number of cells. Three days later, half of the cultures were fed with media containing hydrocortisone sodium succinate at 0.1 μg/ml and the other half were fed with the same medium but without hydrocortisone. This was arbitrarily designated day zero. Cultures were fed with hydrocortisone and control media twice weekly. Starting on day 31, 2 cultures from each set were sacrificed for nuclei count (3), 2 were fed with medium containing palmitate-1-^{14}C, 2 with oleate-1-^{14}C, and 2 with cholesterol-26-^{14}C. Seventy-two hr later, CO_2 was trapped, lipids were extracted, and radioactivity was monitored by procedures described previously (1).

Results. Table I shows the effect of hydrocortisone on the uptake of oleate-1-^{14}C relative to oxidation of oleate-1-^{14}C into $^{14}CO_2$. In control cultures, age-dependent increase in the lipid/CO_2 ratio was consistently observed in all experiments. In hydrocortisone-treated cultures, however, this age-

* Supported in part by Research Grant (HD 00703) from National Institutes on Health.

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TABLE I. Effect of Hydrocortisone on Incorporation and Oxidation of Oleate-1-^{14}C by PHA Cultures.

Expt.	Age of culture (day)	Cell × 10^4/culture		Radioactivity (cpm above background/10^4 cells)					
				Control			Hydrocortisone treated		
		Control	Treated	CO_2	Lipid	Lipid/CO_2	CO_2	Lipid	Lipid/CO_2
1	31	29	205	76	783	10.3	49	218	4.4
	59	17	138	26	373	14.4	108	367	3.4
2	31	31	108	102	725	7.1	72	418	5.8
	59	14	122	70	897	12.8	56	283	5.0
	87	4	47	88	1956	22.8	87	800	9.2
3	31	28	133	147	229	1.6	91	452	4.9
	59	21	214	90	751	8.4	87	301	3.5
	87	12	157	108	2091	19.3	101	435	4.3
	115	6	69	145	3006	20.7	314	871	2.8
4	31	38	137	125	437	3.5	190	277	1.5
	59	15	146	176	913	5.2	182	267	1.5

dependent change was no longer present. Similar results were obtained with palmitate-1-^{14}C in four experiments.

Table II shows the effect of hydrocortisone on the incorporation of cholesterol-26-^{14}C into cell lipids. The age-dependent increase in control culture was again evident in all experiments. Hydrocortisone did not reverse but did slow considerably this trend.

Discussion. Results presented here were the outcome of a long term investigation of the biology of senescence of human cells *in vitro* which had been arbitrarily defined as increase in chronologic age of a population of predominantly nondividing cell (1). That cultures of PHA cell, after 28–56 days *in vitro*, consisted chiefly of nondividing cells for several months had also been documented (4).

Following the procedure described by Geyer (5) for dispersing fatty acid in culture medium, some of the inconsistent results reported previously (1) were no longer encountered in this series of experiments. The importance of assuring adequate dispersion of water-insoluble substrate in culture medium was stressed by Geyer (5). We feel confident that those age-dependent changes in lipid metabolism described previously (1) can be reproduced at will and that these changes can be used as a parameter in studying senescence of PHA cells.

Hydrocortisone is a compound capable of prolonging postmitotic life span of PHA cell, and is also capable of abolishing age-dependent increase in lipid/CO_2 ratio of PHA culture fed with palmitate or oleate and delaying age-dependent increase in incorporation of cholesterol into cell lipids. X-

TABLE II. Effect of Hydrocortisone on the Incorporation of Cholesterol-26-^{14}C into Lipids of PHA Cultures.

Expt.[a]	Age of culture (day)	Radioactivity in lipids (cpm/10^4 cells)	
		Control	Hydrocortisone
1	31	Not done	189
	59	Not done	328
	94	Not done	984
2	31	397	354
	59	425	196
	87	2138	437
3	31	290	225
	59	399	221
	87	1112	410
	115	3014	1180
4	3	142	165
	31	222	222
	59	459	270

[a] Same as corresponding experiments in Table I except that cholesterol-26-^{14}C instead of oleate-1-^{14}C was used as substrate (see Table I for cell number per culture).

irradiation, which is considered by some investigators to be capable of accelerating aging in animals (6), was shown to hasten the age-dependent increase in uptake of cholesterol by PHA cells (7). Changes in lipid/CO_2 from X-irradiated PHA cultures fed with palmitate were inconsistent from experiment to experiment (7). It was possible that this inconsistency might be due to difficulty in dissolving palmitate in nutrient medium. This needs to be reinvestigated. Since the solubility problem is less troublesome with oleate than with palmitate (4), and since similar results were obtained with oleate or palmitate as substrate, it is our opinion that oleate is the preferred substrate for the type of investigation described in this report.

Summary. Hydrocortisone, a compound capable of prolonging postmitotic life span of primary human amnion cells *in vitro* was also capable of abolishing the age-dependent increase in lipid/CO_2 ratio of cultures fed with palmitate or oleate, and of delaying the age-dependent increase in incorporation of cholesterol into cell lipids.

1. Chang, R. S., Arch. Intern. Med. **110**, 563 (1962).

2. Yuan, G. C., Chang, R. S., Little, J. B., and Cornil, G., J. Gerontol. **22**, 174 (1967).

3. Sanford, K. K., Earle, W. R., Evans, V. J., Waltz, H. K., and Shannon, J. E., J. Natl. Cancer Inst. **11**, 773, 1951.

4. Chang, R. S., J. Natl. Cancer Inst. **40**, 491 (1968).

5. Geyer, R. P., Wistar Inst. Symp. Monogr. **6**, (1966).

6. Handler, P., Federation Proc. **20**, Suppl. **8**, (1961).

7. Yuan, G. C., Little, J. B., and Chang, R. S., J. Gerontol. **20**, 522 (1965).

Received Oct. 11, 1968. P.S.E.B.M., 1969, Vol. 130.

Study of Immunoglobulins in Axenic Mice Thymectomized at Birth* (33692)

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In several strains of holoxenic mice, neonatal thymectomy is followed during early life by alterations of serum immunoglobulins (1–3), growth, some immune functions (4–8) and reticuloendothelial phagocytic activity (9). These troubles are associated in varying degrees with a wasting syndrome. As most of these consequences of neonatal thymectomy do not appear in axenic mice (10–12), we studied immunoglobulins in axenic C3H/J mice thymectomized at birth. The aim of the present experiment was to evaluate the respective influence of the thymectomy itself and of the secondary neonatal and chronic infection on the levels of immunoglobulins.

Materials and Methods. Axenic C3H/J young mice were thymectomized within 24 hr of birth, according to Wilson's technique (13). The animals were kept in flexible polyvinyl chloride isolators and fed a Labena special diet (Duquesne Purina) and sterilized water. Between the ages of 1 and 8 months some of the animals were taken out of the isolators and bled by the orbital sinus; others were bled within the isolators in the same manner. Sham thymectomized, and nonoperated axenic C3H/J mice holoxenic C3H/J of the same age were bled for control. The experiment was performed on 72 thymectomized animals, 14 sham thymectomy and 94 control animals. Some of the mice were bled only once, while others were bled 2 or 3 times.

White blood cell counts were performed on some of the blood samples (25 thymectomized, 26 control).

A certain number of the animals were killed after the bleeding and their thymic site

* Supported by Fondation pour la Recherche Médicale Française grant.