inhibitor of estrus and conception in the female rat; in addition, it does not interfere with mating, nor have permanent effects on fertility.

Enol etherification by cyclopentylic alcohol, while reducing the anti-estrual and contraceptive activity of MAP, enhances those of ENTA. It is possible that these changes of activity reflect parallel changes in other biological properties; however, the progestational potency of MAPc-5 and of ENTAc-5 as judged by the Clauberg test, and of MAPc-5 as shown by the pregnancy maintenance test in ovariectomized rats†, appears to remain practically unmodified in comparison with their respective parent compounds. Enol etherification of both MAP and ENTA produces a striking dissociation in the contraceptive and progestional activities of the parent compounds; MAPc-5 is a less active contraceptive and anti-estrual agent than MAP, while ENTAc-5 is 4 times as active as ENTA in this respect. On the other hand, enol etherification of AP neither enhances nor decreases any inhibitory effects on estrus, mating or fertilization within the limits of the experiment.

Summary. Cyclopentyl enol ethers of 17α-acetoxyprogesterone, 6α-methyl-17α-acetoxyprogesterone and 17α-ethynyl-19-nortestosterone acetate were tested for ability to inhibit estrus, mating and fertilization in female rats with the following results: The first compound showed no inhibitory effect; the second blocked the estrous cycle, without interfering with mating and fertilization; the third proved to be a potent antiestrual and contraceptive agent, although it did not disturb sexual receptivity or cause permanent sterility. Thus, enol etherification reduces the antifertility effects of 6α-methyl-17α-acetoxyprogesterone, but strongly enhances those of 17α-ethynyl-19-nortestosterone acetate.

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† Unpublished observations (Falconi, G.)

1. Ercoli, A., Gardi, R., *J. Am. Chem. Soc.,* 1960, v82, 746.

2. Ercoli, A., Bruni, G., Falconi, G., Galletti, F., Gardi, R., *Acta Endocr.* (Kbh), Suppl. 51, 1960, v35, 857.

3. Ercoli, A., Bruni, G., Falconi, G., Gardi, R., Meli, A., *Endocrinology,* 1960, v67, 521.

4. Falconi, G., Gardi, R., Bruni, G., Ercoli, A., *ibid.,* in press.

5. Lahr, E. L., Riddle, O., PROC. SOC. EXP. BIOL. AND MED., 1936, v34, 880.

6. Phillips, W. A., *Am. J. Physiol.,* 1937, v119, 623.

7. Slechta, R. F., Chang, M. C., Pincus, G., *Fertil. and Steril.,* 1954, v5, 282.

8. Velardo, J. F., *Ann. N. Y. Acad. Sci.,* 1958, v71, 542.

9. Sala, G., Baldratti, G., Arcari, G., *Moderne Entwicklungen auf dem Gestagengebiet,* H. Nowakowski (Editor), Springer, Berlin, 1960, p58.

10. Ingram, D. L., *J. Endocrinol.,* 1959, v19, 182.

11. Boling, J. L., Blandau, R. J., Rundlett, B., Young, W. C., *Anat. Rec.,* 1941, v80, 155.

12. Mandl, A. M., *J. Endocrinol.,* 1959, v18, 434.

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Comparison of Lipid Metabolism of Chicken Embryo Organs and Cells in Culture.* (26828)

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Differences exist in amino acid and carbohydrate metabolism(1,2) between primary cultures and established cell lines. Little is known of the relationship between the metabolism of primary cultures and parent organs. Therefore, a comparison of lipid me-

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tabolism of chicken embryo organs and cells in culture was made. Since at an embryonic stage, cell proliferation is very rapid, conditions may be similar to those that prevail in cell cultures. Incorporation of acetate-1-C^{14} into lipids was studied in cells of chicken embryo *in vivo* and in tissue culture, and also in HeLa and liver (Chang) cells. The isotope concentrations of cholesterol esters, neutral fats, and phospholipids were compared and used as a measure of the metabolism of the lipid fractions.

Materials and methods. The basal medium consisted of Hanks' balanced salt solution with addition of amino acids, vitamins and antibiotics(3). To cultures prepared from chicken embryo 0.1% of lactalbumin hydrolysate (Sheffield Chemicals) was added to stimulate growth. These cells were grown in presence of 2.5% chicken or calf serum (Microbiological Associates, Inc.). HeLa and liver (Chang) cells were cultivated in the same basal medium with addition of 10 or 5% horse serum respectively. Sodium acetate-1-C^{14} (specific activity: 1 millicurie/41 mg (New England Nuclear Corp.) was added to furnish 0.02 mc/100 ml complete medium. For each determination 2-3 bottles of cells were pooled. Each experiment consisted of 3 separate determinations.

Inocula of primary cultures were prepared from organs of 12-day-old chick embryos, as described by Neuman *et al.*(4). Each 250 ml bottle was inoculated with 10 ml of medium containing 4×10^5 cells per ml. After a 3-day period at 37°C the cells were washed with physiological saline, scraped, and washed again.

Inocula of HeLa and liver cells consisted of 10 ml of medium containing about 6×10^4 cells per ml. According to the amount of growth, cells were scraped after 3-4 days, and thereafter treated as described above.

In a manner similar to that described by Marrian *et al.*(5), 0.5 ml acetate-1-C^{14} solution (0.4 mc/10 ml saline) was injected into the fertilized egg air sac under aseptic conditions either on the 9th or 11th day of incubation. On the 12th day, the embryo organs were isolated, washed and extracted for lipids. Preliminary experiments had shown that 5 mg sodium acetate per egg could be tolerated without apparent damage to the embryo. Each determination was done by pooling organs from at least 3 embryos. Each experiment consisted of 3 separate determinations.

Lipids of washed cells or organs were extracted and washed several times as described by Folch, *et al.*(6). Lipid fractions were separated on silicic acid columns(7) into: 1. cholesterol esters; 2. neutral fats, free fatty acids, and cholesterol; and 3. phospholipids. The neutral fat fraction was rechromatographed on short alumina columns(8). Free cholesterol present in fraction 2 was evaluated, using the digitonin method(9). C^{14} activities of various fractions were measured as ultrathin specimens with a thin-window Geiger tube. Serum fatty acids were analyzed using a gas chromatographic method (10). Nitrogen was determined with Nessler reagent(11), serum cholesterol according to the method of Abell *et al.*(12), and total serum fatty acids by the Albrink method(13).

Results. When fertilized eggs were injected with acetate-1-C^{14} and incubated either 24 or 72 hours, the relative C^{14} incorporation in embryonic tissues was greatest in phospholipids (>75%), and less in neutral fats and cholesterol esters (Table I). Total incorporation was maximal in stomach, less in thigh, and still less in heart, and was higher after the shorter incubation period. Actual C^{14} content of the cholesterol ester fractions of the 3 organs was fairly constant, but based on relative distribution within the lipids of a given tissue the cholesterol ester fraction of the heart was highest and that of stomach lowest.

In experiments employing cell cultures from chick embryo heart, thigh, and stomach more than 70% of lipid radioactivity was present in the neutral fat fraction, with most of the remainder in the phospholipids, and a small percentage in the cholesterol ester fraction (Table I). Total radioactivity/mg protein was considerably higher in lipids of thigh and stomach grown in presence of calf serum, than when chicken serum was present. The relative percentage of C^{14} in the cholesterol ester fraction was significantly higher for all

TABLE I. Incorporation of Acetate-1-C^{14} into Cholesterol Esters (CE), Neutral Fats (NF), and Phospholipids (PL) in Chicken Embryo Organs and Cultures Derived from Them.

Organ	Lipid fractions	Relative C^{14} distribution: Organs 24 hr*	Organs 72 hr*	Cultures 72 hr* Chicken serum	Cultures 72 hr* Calf serum
		%			
Heart	CE	3.1 ± .06†	4.3 ± .7	2.8‡(2.0- 3.6)	.8‡(.8- .8)
	NF	20.5 ± 1.9	20.3 ± .7	81.9 (80.5-83.2)	80.6 (75.5-85.7)
	PL	76.4 ± 1.9	75.4 ± .3	15.4 (13.2-17.5)	18.6 (13.5-23.7)
	Total cpm/mg protein	346	155	14,487	13,090
Thigh	CE	1.7 ± .35	2.8 ± .47	1.9 ± .20	.9 ± .0
	NF	13.3 ± .5	16.8 ± .4	75.9 ± 3.9	65.0 ± 1.7
	PL	84.9 ± .7	80.4 ± .2	22.1 ± 4.1	33.9 ± 1.7
	Total cpm/mg protein	995	344	11,855	19,383
Stomach	CE	.9 ± .07	1.1 ± .17	4.8 ± .18	2.2 ± .46
	NF	15.8 ± 1.4	17.0 ± .6	81.3 ± .3	78.3 ± 2.6
	PL	83.2 ± 1.4	81.9 ± .7	13.9 ± .9	19.5 ± 2.6
	Total cpm/mg protein	1,352	572	11,882	18,580

* Period of incubation with acetate-1-C^{14}.
† Mean ± stand. error.
‡ Two determinations. Two pools of 3 bottles each were used.

3 tissues grown in chicken serum as compared with calf serum. As judged by a *T* test(14) the significance of these differences between the means for thigh and muscle was $P<0.01$. Radioactivity due to free cholesterol and unesterified fatty acids in the neutral fat fractions was negligible.

The radiocarbon content of the lipids of 2 established cell lines, HeLa and liver (Chang), grown in presence of acetate-1-C^{14} was highest in phospholipids, lower in neutral fat, and very low in the cholesterol ester fractions (Table II).

Discussion. Lipid C^{14} of chicken embryo organs from fertilized eggs injected with acetate-1-C^{14} was present predominantly in phospholopids. These results resembled those obtained with rats fed acetate-1-C^{14} for 24 or 72 hours in which most lipid C^{14} was recovered in phospholipids(15). In cells cultured from chicken embryo organs, however, neutral lipids contained most of the lipid C^{14}. Radiocarbon distribution in lipids of HeLa and liver (Chang) cells was similar to that of conjunctival (Chang) cells(16). Marks *et al.*(17) found radioacetate mainly incorporated into neutral lipids of normal leukocytes, but equally into neutral fats and phospholipids of leukemic cells. Therefore, of cells so far studied *in vitro* only those cells which were non-malignant or incapable of unlimited rapid and autonomous multiplication, incorporated most of the radioacetate into neutral fat. Further, from the standpoint of acetate incorporation, such cells bore the least metabolic resemblance to metabolism *in vivo*.

TABLE II. Isotope Content of HeLa and Liver Cell Lipids.

Fraction	% of C^{14} concentrations HeLa*	Liver*
Cholesterol esters	.4 ± .06†	.5 ± .15
Neutral fats	42.9 ± 1.9	43.0 ± .9
Phospholipids	56.7 ± 1.8	56.5 ± 1.6
Total cpm/mg protein	27,507	23,417

* HeLa cells were grown for 3 days; liver cells for 4 days.
† Means and stand. errors.

Exogenous lipids gain access to tissue culture cells(18,19); however, the influence of kind of serum on relative incorporation of acetate into cholesterol esters (Table I) cannot be explained readily on the basis of degree of unsaturation, fatty acid composition, or total fatty acid and cholesterol content (Table III). This is in agreement with

TABLE III. Fatty Acid and Cholesterol Composition of Chicken and Calf Serum.*

Fatty acid		% composition	
No. of carbons	Degree of saturation	Chicken	Calf
C_{12}	saturated	trace	.5
C_{14}	"	.4	2.5
C_{15}	"	.1	.9
C_{16}	"	12.1	11.9
C_{16}	monoenoic	2.0	3.6
C_{18}	saturated	19.5	14.2
C_{18}	monoenoic	32.0	24.8
C_{18}	dienoic	25.6	24.7
C_{18}	trienoic	.4	8.8
C_{20}	tetraenoic	5.9	3.8
Unsaturated fatty acids (%)		65.9	65.7
Saturated " " (%)		32.1	30.0
Total cholesterol (mg %)		138.0	152.0
" fatty acids (meq/l)		18.3	24.4

* Based on the gas chromatography(14) of normal and hydrogenated methyl esters using 20% Craig polyester succinate on 60-80 mesh Chromosorb W (Wilkens Instrument and Research, Inc., Walnut Creek, Calif.) at 180°C and argon flow rate of 60 ml/min. A Research Specialties Co. instrument equipped with a strontium ionization detector was used. Small amounts of other acids were present, but comprised less than 5% of the total and were omitted from the results.

Bailey(20) who showed cholesterol uptake of mouse lymphoblasts (MB III) was influenced by the kind of serum used, but not by the cholesterol or total fatty acid content of the serum itself. He concluded that the relationship between serum cholesterol and the binding power of the serum proteins may be the most important factor. No influence of exogenous stearic or linoleic acids was noted. Further studies are required to elucidate fully the mechanism(s) of the action of serum. Primary cultures, such as those of chicken embryos, as well as established cell lines, should be of value in studies of cholesterol ester and lipid metabolism as affected by various drugs and nutrients.

Summary. A study was made of the relative incorporation of acetate-1-C^{14} into lipids of chicken embryo tissues *in ovo* and in tissue culture. Embryonic heart, thigh, and stomach incorporated C^{14} mainly into phospholipids *in ovo,* and into neutral fat *in vitro.* Relative C^{14} content of the cholesterol esters was higher when the cells were grown in medium containing calf serum as compared with chicken serum, an effect not correlated with serum fatty acid composition or fatty acid and cholesterol content. Acetate incorporation by established cell lines (HeLa and Chang liver) resembled more closely that of chick embryo organs *in ovo* rather than that of chick embryo tissues *in vitro.*

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1. Pasieka, A. E., Morgan, J. F., *Nature,* 1959, v183, 1201.

2. Chang, R. S., *J. Exp. Med.,* 1961, v113, 405.

3. Neuman, R. E., McCoy, T. A., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 303.

4. Neuman, R. E., Tytell, A. A., *ibid.,* 1960, v103, 763.

5. Marrian, D. H., Hughes, A. F. W., Werba, S. M., *Biochim. et Biophys. Acta,* 1956, v19, 318.

6. Folch, J., Lees, M., Stanley, G. H. S., *J. Biol. Chem.,* 1957, v226, 497.

7. Portman, O. W., Pinter, K., Hayashida, T., *Am. J. Clin. Nutr.,* 1959, v7, 63.

8. Fillerup, D. L., Knauf, J. W., Mead, J. F., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 862.

9. Merrill, J. M., *Circulation Res.,* 1959, v7, 709.

10. Farquhar, J. W., Insull, W., Rosen, P., Stoffel, W., Ahrens, E. H., *Nutr. Revs.* (Supplement), 1959, v17, Part II, 1.

11. Lang, C. A., *Anal. Chem.,* 1958, v30, 1692.

12. Abell, L. L., Levy, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

13. Albrink, M. J., *J. Lipid Res.,* 1959 v1, 53.

14. Snedecor, G. W., *Statistical Methods,* Iowa State College Press, Ames, 1959.

15. Pihl, A., Block, K., *J. Biol. Chem.,* 1950, v183, 431.

16. Geyer, R. P., Sperry, M., Sholler, R., *Proc. Am. Assn. Cancer Res.,* 1961, v3, 227.

17. Marks, P. A., Gellhorn, A., Kidson, C., *ibid.,* 1961, v3, 248.

18. Bailey, J. M., Gey, G. O., Gey, M. K., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 686.

19. Geyer, R. P., Neimark, J. M., *Am. J. Clin. Nutr.,* 1959, v7, 86.

20. Bailey, J. M., Martyn, J., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 30.

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