two factors are completely corrected by Stypven. The finding that Stypven does not correct the clotting defect of Stuart factor deficient plasma implies that certain modified one-stage "specific" assay methods for prothrombin using Stypven as a source of factor VII are not, in fact, specific, and measure both prothrombin and Stuart factor.

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1. Lewis, J. H., Fresh, J. W., and Ferguson, J. H., PROC. SOC. EXP. BIOL. AND MED., 1953, v84, 651.
2. Alexander, B., Goldstein, R., Landwehr, G., and Cook, C. D., *J. Clin. Invest.*, 1951, v30, 596.
3. Koller, F., Loeliger, A., and Duckert, F., *Acta Hemat.*, 1951, v6, 1.
4. Owren, P. A., *Scand. J. Clin. and Lab. Invest.*, 1951, v3, 168.
5. Duckert, F., Fluckiger, P., Matter, M., and Koller, F., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 17.
6. Hicks, N. D., *Med. J. Aust.*, 1955, v2, 331.
7. Graham, J. B., and Hougie, C., *Proc. 6th Internat. Conf. Hemat.*
8. Jenkins, J. S., *J. Clin. Path.*, 1954, v7, 287.
9. Rapaport, S. I., Aas, K., and Owren, P. A., *Blood*, 1954, v9, 1185.
10. Biggs, R., and Douglas, A. S., *J. Clin. Path.*, 1953, v6, 23.
11. Bell, W. N., and Alton, H. G., *Nature*, 1954, v174, 880.
12. Ratnoff, O. D., and Colopy, J. E., *J. Clin. Invest.*, 1955, v34, 602.
13. Rosenthal, R. L., *Am. J. Med.*, 1954, v17, 57.
14. Spaet, T. H., Aggeler, P. M., and Kinsell, B. G., *J. Clin. Invest.*, 1954, v33, 1095.
15. Telfer, T. P., Denson, K. W., and Wright, D R., *Brit. J. Haemat.*, 1956, v2, 308.
16. Hjort, P., Rapaport, S. I., and Owren, P. A., *J. Lab. Clin. Med.*, 1955, v46, 89.
17. Owren, P. A., and Aas, K., *Scand. J. Clin. and Lab. Invest.*, 1951, v3, 201.

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Oxygen Uptake and Lactate Formation of HeLa Cells.*† (22824)

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HeLa cells were obtained from an epidermoid carcinoma of the cervix and started as a tissue culture in 1951(5). After 4 years *in vitro*, smears stained by Papanicolou's technic (4) indicated that HeLa had malignant features. It has been grown by Toolan in cortisone-treated rats and in human subjects in terminal stages of another neoplastic process (5). The cells are uniform in appearance as compared to mixed cell types found in tissue. They can be easily separated without apparent injury. Cell number or weight can be determined quite accurately since there is little interstitial material. For these reasons HeLa makes an ideal cell suspension on which to study metabolism. Our primary objective was to compare metabolism of human HeLa cells to that reported characteristic of animal tumors(2). Originally Gey started HeLa in medium which contained human serum. Other laboratories§ adapted a line of HeLa cells to grow in medium containing horse serum. Our second objective was to compare metabolism of cells cultured in human serum‖ with that of cells cultured in horse serum.

Procedure. At start of this study 2 lines of HeLa were carried in medium containing 40% Earle solution, 20% chick embryo extract (50:50) and 40% horse or human serum. It was desired to carry one line of cells

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§ Original cultures obtained from Dr. W. R. Earle.

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in human medium. Subcultures of each line were placed in semi-synthetic medium which had proven adequate for indefinite growth of Earle's Strain L cells. An initial solution contained 0.25 g yeast¶ and 0.25 g lactalbumin hydrolysates/liter Earle solution. Horse or human serum (10%) was added before use. Culture medium was changed 3 times weekly and all metabolic determinations made the day following a culture change. Determinations on HeLa were made in 2 suspending solutions. In one, phosphate solution alone was used, in the other 10% neutralized horse or human serum(10) was added to it. Respiration was determined by direct method of Warburg(9) which does not allow use of Earle solution because of its high bicarbonate content. Phosphate buffered solution (pH 7.4) adopted for use, contained the following materials per liter: 8.12 g. NaCl, 0.28 KCl, 0.15 $CaCl_2$, 0.33 $MgSO_4 \cdot 7\ H_2O$, 0.28 Na_2HPO_4, 0.05 $NaH_2PO_4 \cdot H_2O$, 1 Glucose, 0.25 lactalbumin hydrolysate, 0.25 yeast hydrolysate. Lactate was determined chemically by the method of Barker and Summerson(1). Concentration in one reaction vessel was obtained immediately after equilibration for temperature and in another an hour later. Difference between concentrations in the 2 vessels was taken as lactate formation. During experimentation, number of HeLa cells in a reaction vessel was determined by counting on a hemacytometer. To obtain dry weight intercellular material was digested with 0.25% Difco trypsin (1:250) in Earle solution. The cells were collected by centrifugation, resuspended in Earle solution, counted, then collected again and dried at 110°C to constant weight. Three determinations indicated that one million HeLa cells weighed 0.99 mg. Phenylenediamine was used to obtain a measure of functional cytochrome reserve. This was done by adding p-phenylenediamine from side arm of reaction vessel so that final concentration was 0.02 M. Respiration was then compared with that in control flask without p-phenylenediamine. In preliminary respiration experiments, 2 methods of separating cells were tried. Trypsin was used in one method. In the other, cells were brushed from floor of T-60 flasks with brush made of stainless steel and nylon bristles and separated further by filling and discharging them through a pipette 15 times. Since trypsin appeared to depress respiration in some cases (Table I), use of nylon brush was considered the better method for cell separation. It is necessary to assume a constant rate of oxygen uptake for a definite interval to express Qo_2 values as mm^3/hr. Rate of oxygen uptake for practical purposes was constant for one hour, but slightly less at 2 hours (Fig. 1). Subsequent determinations were run for exactly one hour. Respiration was higher at 38°C than at 37.5°C ($P<.01$) but the lower temperature was used for metabolic determinations because incubators used for growing cultures had been maintained at 37.5°C.

TABLE I. Effect of Separation Methods on Respiration of HeLa.

Method	Culture serum	Warburg sol.	Temp., °C	Qo_2	P
Brush	Horse	Saline	37.5	2.2 (8)	
Trypsin			37.5	2.2 (5)	
Brush	Horse	Serum	38.0	7.7 (2)	>.05
Trypsin			38.0	5.8(13)	
Brush	Human	Saline	37.5	8.4 (6)	<.05
Trypsin			37.5	4.7 (5)	

Figures in parentheses indicate No. of determinations.

Results. Comparison was made either between quotients obtained in saline or between those in saline-serum. Without exception, all metabolic quotients were higher for HeLa cells grown in medium containing human se-

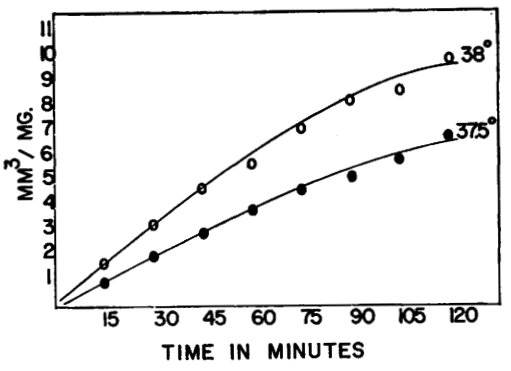

FIG. 1. Oxygen uptake of HeLa in neutralized horse serum.

¶ Nutritional Biochemicals Corp.

TABLE II. Respiration and Glycolysis at 37.5°C of HeLa Grown in Media Containing Either Human or Horse Serum.

Culture serum	Warburg solution	Q_{O_2}	$Q_{LA}^{O_2}$	$Q_{LA}^{N_2}$	P-PD
Horse	Saline	2.2 (13)	6.7 (3)	3.2 (2)	11.9 (3)
Human	"	6.3 (11)	10.2 (12)	8.4 (6)	14.3 (5)
Horse	" + horse serum	4.0 (13)	7.2 (4)	5.2 (5)	21.7 (4)
Human	" + human "	6.0 (4)	12.0 (4)	5.5 (5)	26.3 (4)

All values expressed as mm^3/mg dry wt/hr. Figures in parentheses indicate No. of determinations. P-PD = Q_{O_2} when 0.02 M p-phenylenediamine added.

rum than those grown in a horse serum medium (Table II).

Quotients determined in saline and in serum were about the same order of magnitude. There were exceptions. Respiration of HeLa adapted to grow in horse serum, when determined in saline, was lower than similar determinations in horse serum ($P<.05$). Phenylenediamine stimulated respiration of cells in solution containing serum to a greater extent than it did when saline alone was used; this was true for cultures which had been grown in horse serum ($P<.01$) or human serum ($P<.05$).

It has been pointed out by Warburg, Burk and others(2,9) that tumors have a metabolism that differs from normal tissues. HeLa cells, carried by tissue culture technic, metabolized in some ways like *in vivo* derived tumor slices but in other ways like normal tissue(6) (Table II). Metabolism of HeLa cells was similar to that reported for tumors, in that respiration (Q_{O_2}) was moderately low and aerobic lactic acid formation ($Q_{LA}^{O_2}$) moderately high. They exhibit metabolism dissimilar to that reported for tumors in that anerobic lactic acid formation ($Q_{LA}^{N_2}$) was low and added p-phenylenediamine stimulated respiration greatly (338%). The latter observation was indirect evidence that adequate functional cytochrome reserve was present. There was no Pasteur Effect ($Q_{LA}^{N_2} - Q_{LA}^{O_2}$) because anerobic glycolysis was not in excess of aerobic glycolysis. Respiration and anerobic glycolysis were about the same magnitude and thus Fermentation Excess ($Q_{LA}^{N_2} - 2Q_{O_2}$) was negative.

Discussion. Numerous workers have shown that animal cells grown in chemically defined medium need, among other things, certain amino acids and vitamins. In an attempt to obtain a simple homologous medium enzymatic lactalbumin hydrolysate was used because Melnick and Riordon(8) had found it to be growth stimulating; enzymatic yeast hydrolysate was used so that most B vitamins as well as amino acids would be present. Hydrolysates were added to the medium in concentration that would yield an amino acid solution very similar to that found in serum (7). The medium was sterilized by Selas filtration rather than by autoclaving(8) so that thiamin, folic acid and vit. B_{12} would not be destroyed. Preliminary results indicated that hydrolysate culture medium, as used in this study, compared favorably to Eagle's synthetic medium(3) supplemented with 10% serum.

One early observation which we did not readily accept was that aerobic glycolysis was higher than anerobic glycolysis even when bound bicarbonate was present in neutralized serum. Our repeat experiments gave similar results. Warburg(9) has shown that glycolysis of more than 20 different tumors was higher under anerobic than aerobic conditions. It is interesting to note that Hellerman collaborating with Gey(5) found that aerobic lactate was higher than anerobic lactate for the 14P rat fibroblast cultured *in vitro*.

Summary. Gey's strain HeLa cells were cultured in Earle T-60 flasks for almost a year in media composed of 0.25 g. yeast and 0.25 g. lactalbumin hydrolysate/l Earle solution with 10% human or horse serum. Respiration was determined manometrically and lactate formation chemically. In preliminary experiments, 0.25% Difco trypsin (1:250) when used to separate cells, depressed respiration in some instances. Respiration of sus-

pensions of HeLa was constant for an hour but decreased slightly after first hour. Metabolism of two lines of HeLa cells was compared. Respiration and glycolysis of cells grown in media containing homologous human serum was higher than that of cells grown in media containing horse serum. Human HeLa cells had a metabolism like that reported for tumor tissue in that respiration was low ($Q_{O_2} = 6.0$) and aerobic glycolysis moderate ($Q^{O_2}_{L.A} = 12.0$). They differed from tumor tissue in that anerobic glycolysis was low ($Q^{N_2}_{L.A} = 5.5$) and p-phenylenediamine stimulated respiration 338% ($Q_{O_2} = 26.3$). There was no Pasteur Effect and Fermentation Excess was negative.

1. Barker, J. B., and Summerson, W. H., *J. Biol. Chem.*, 1941, v138, 535.
2. Burk, D., Earle, W. R., Winzler, R. J., *J. Cancer Inst.*, 1944, v4, 363.
3. Eagle, H., *Science*, 1955, v122, 501.
4. Fennell, R. H., *Anat. Rec.*, 1956, v124, 10.
5. Gey, G. O., *The Harvey Lectures*, New York, Academic Press, 1954-55, Series L, 154.
6. Greenstein, J. P., *Biochemistry of Cancer*, New York, Academic Press, 1954, 451.
7. Krebs, H. A., *Ann. Rev. Biochem.*, 1950, v19, 409.
8. Melnick, J. L., and Riordon, J. T., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 208.
9. Warburg, O., *The Metabolism of Tumors*, London, Constable and Co., 1930.
10. Warren, C. O., *J. Biol. Chem.*, 1944, v156, 559.

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Absence of Appreciable Resistance to Growth Inhibiting Action of Cortisone After Prolonged Treatment.* (22825)

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Chronic administration of nitrogen retaining hormones, to the plateaued adult Norway rat, leads to period of rapid growth followed by attainment of a new weight plateau on which there is no further growth although dosage of hormone per animal or per unit weight, is unchanged. This phenomenon of plateauing on chronic treatment with anabolic hormones appears to be identical in protein anabolic steroids(1,2) in anterior pituitary growth hormone(3-5); and in anabolic steroids plus growth hormone(6). Glucocorticoids have been shown to have a striking growth inhibiting action(7,8) due to their protein catabolic action. It would, therefore, be of interest to determine if there is a failure of constant dosage of glucocorticoid to give a constant inhibition of growth; indeed, the data of Tolksdorf *et al.*(8) suggest that this might be the case.

The present investigation was performed to further clarify possible development of resistance to growth inhibiting action of glucocorticoids. Since the time required for growth cessation, on a constant dosage of anterior pituitary growth hormone, is highly sensitive to the dose level(9), a range of dose levels was employed.

Methods. A microcrystalline suspension of cortisone acetate† (Δ^4-Pregnene-17β, 21-diol-3,11,20-trione-21-acetate) in isotonic saline was prepared by trituration with Tween-80 (polyoxyethylene sorbitan monooleate). Concentration of the suspension was adjusted to contain 3.2 mg cortisone/ml and less than 0.2 % Tween-80. After dilution, the suspension was sealed in injection vials and stored at 2 to 4°C until just before injection. Single daily subcutaneous injections were given under strictly aseptic conditions, at systematically varied sites along dorsal surface of the rat. Dosage was adjusted every 2 days

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