to react in these tests. The principles applied to attain satisfactory formalinized cells are discussed.

The author wishes to thank Roland Mills for technical assistance, and Dr. C. W. Hiatt for the electron micrographs.

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Received October 21, 1959. P.S.E.B.M., 1960, v103.

Instability of Metabolic Quotients Obtained from Tissue Cultures.* (25445)

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To compare the metabolism of one tissue culture with that of another they should be maintained under conditions that yield reproducible results. Serum in tissue culture medium introduces a variable. Since serum is not chemically defined, much work has been done to devise synthetic growth media. Some tissue cultures have been grown in absence of serum(3,4) but these conditions are the exception rather than the rule, because most cell strains must still be maintained in serum. Although use of serum in nutritional studies can be questioned, there has been little objection to its use for growing cells for subsequent metabolic determinations, especially if cells are washed and resuspended in a chemically defined solution before the determination is made. We noted previously(7) that HeLa cells grown in medium containing human serum respired and formed lactate at a greater rate than the same cells grown with horse serum. These observations suggested the possibility that tissue cells grown in serum from different members of the same species may respire and form lactate at different rates.

Materials and methods. Earle's Strain L cells were grown in saline solution containing 0.5% enzymatic lactalbumin hydrolysate plus 20% horse serum. Cultures were gassed with 5% CO_2 in air. Saline solution contained in g liter: NaCl 6.5, KCl 0.29, $MgSO_4 \cdot 7H_2O$ 0.21, $CaCl_2$ 0.14, $NaHCO_3$ 2.06, Na_2HPO_4 0.15, glucose 2, phenol red 0.05, lactalbumin 5. This and other solutions used, with the exception of trypsin, contained inorganic salts in about the same ratio as in interstitial fluid (12) and had a tonicity equivalent to 0.93% sodium chloride solution as determined by freezing point depression. All solutions were buffered at pH 7.2. At the start of experimentation, cells were grown in 1.9, 7.5, 15, 20, and 30% horse serum to determine optimal serum concentration for growth. Media was renewed every third day and after 10 days the live cells in each culture counted(8). These results graphed in Fig. 1 indicate that 15-20% horse serum plus lactalbumin medium was optimal for growth of Strain L cells. To prepare cells for metabolic determination, they were freed from T-60 culture flasks(2) with

* Supported by grants from Nat. Cancer Inst. and Nebraska Heart Assn. Preliminary report presented at Am. Physiol. Soc. Meeting, Urbana, Sept. 1959.
Technically assisted by Vera Skank.

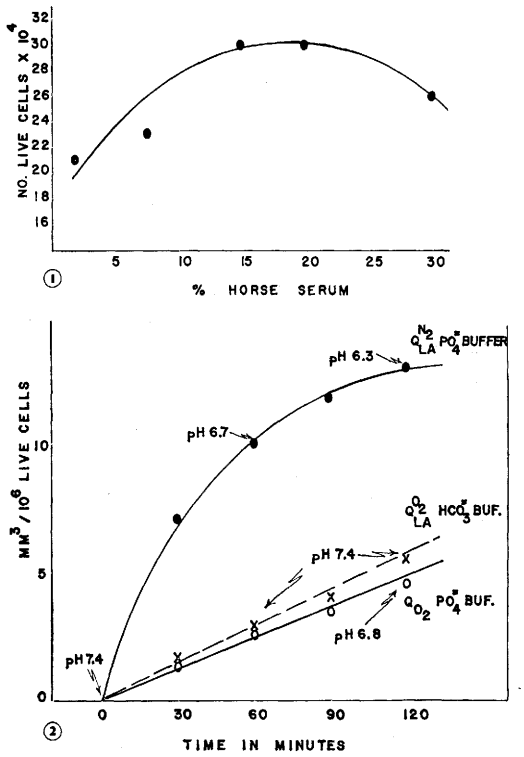

FIG. 1. Effect of horse serum concentration on growth of Strain L cells. Each point represents mean population of 4 roller tube cultures.

FIG. 2. Effect of pH on rate of lactate formation and oxygen uptake. $Q^{N_2}_{LA}$ and $Q^{O_2}_{LA}$ are graphed so that lines do not overlap; rate of both decreases as pH becomes lower.

trypsin on day following renewal of culture medium. The trypsin solution contained 2.5 g trypsin (Nutritional Biochemical Corp. 1:300) in one liter of 0.9% NaCl, and was buffered with 0.29 g Na_2HPO_4 and 0.04 g $NaH_2PO_4 \cdot H_2O$. Cells were dispersed by passing them back and forth through a syringe. They were then collected by centrifugation and half of the cells resuspended in a bicarbonate buffered solution and half in one buffered with phosphate. The bicarbonate solution contained in g/liter: NaCl 7.03, KCl 0.29, $MgSO_4 \cdot 7H_2O$ 0.21, glucose 2, $CaCl_2$ 0.14, Na_2HPO_4 0.15, $NaHCO_3$ 2.06 and gelatin 5. The phosphate solution contained: NaCl 8.42, KCl 0.29, $MgSO_4 \cdot 7H_2O$ 0.21, glucose 2, $NaH_2PO_4 \cdot H_2O$ 0.04, $CaCl_2$ 0.14, Na_2HPO_4 0.31 and gelatin 5. Gelatin was added as a protective agent to each solution because it yielded about 25% more live cells(5) than saline alone. The bicarbonate solution was used to suspend cells for determining lactate. Reaction vessels were gassed with 5% CO_2, either in air or in nitrogen. Lactate was determined chemically as previously described(9). Under these conditions pH 7.2 was held constant which was necessary because it had been found that lactate formation varied greatly with slight changes in pH(9). Respiration was not affected by slight changes of pH as long as pH remained within a range not harmful to tissue cultured cells(8). Results that illustrate the effect of pH change on glycolysis and respiration are graphed in Fig. 2. As pH in phosphate buffered saline decreased due to acid formation, rate of lactate formation also decreased; however, if pH was held constant with strong bicarbonate buffer, lactate formation continued as a straight line function with time. Rate of oxygen uptake in phosphate buffered saline did not change even though pH decreased. As a result of these observations glycolysis was measured in bicarbonate and respiration in phosphate buffered saline in an atmosphere of air by the Direct Method of Warburg(10). All determinations on the Warburg respirometer were made at 37.5°C and metabolic values expressed in terms of live cell counts, made as previously described(8).

Results. Strain L cells which had been grown for 30 days in medium containing horse serum A, had a stable rate of respiration and lactate formation. These results are shown in Table I for 30th and 31st day of experiment. On 32nd day, the cells were changed to a medium containing horse serum B. After the cells had been grown in this serum for 19 days, lactate formation in air was greatly increased; aerobic glycolysis was about the same as anerobic glycolysies. Thirty days after cells had been in horse serum B, respiration and lactate formation stabilized, but at a rate higher than observed in horse serum A. Starting on 67th day the experiment was repeated by growing cells in serum C and similar results were obtained.

These results indicate that respiration and glycolysis of tissue cultured cells may change when culture medium serum is changed. This

TABLE I. Change of Strain L Cell Metabolism with Change in Culture Media Horse Serum.

Day of exp.	Serum in medium	Amt (l)	Days in serum	Q_{O_2}	$Q_{LA}^{O_2}$	$Q_{LA}^{N_2}$
30	A	9-10	30	1.4	4.6	12.8
31	A		31	1.6	3.1	10.9
50	B	8	19	1.4	18.7	20.7
61	B		30	2.7	8.1	17.1
66	B		35	2.8	9.5	23.2
81	C	4	15	.5	18.3	19.6
97	C		31	3.5	10.0	23.5
98	C		32	3.7	11.0	27.2

Q = $mm^3/10^6$ live cells/hr. Q_{O_2}—oxygen uptake, mean of 12 determinations. Q_{LA}—lactate, mean of 4 determinations.

occurs even though serum is from the same species of animal, and when cells are washed and resuspended in saline before a metabolic determination is made. A change in metabolism by changing culture serum, is first reflected by an increase in aerobic lactate formation which is out of proportion to respiration or anerobic lactate formation. After the cells have been grown in a particular serum for about 30 days, adaptation appears to occur and metabolism stabilizes at a new level.

Cells had to be grown in a particular serum to change the metabolism. This can be shown in another way than that described above. Cells grown in serum C respired for several hours at the same rate even after they were washed and resuspended in either saline, serum C or serum A (Table II). These results also indicate that respiration, as measured, is not immediately affected by possible metabolite differences of the 2 sera.

Rate of oxygen uptake is related to growth rate. If growth rate is lowered by reducing incubator growth temperature to 31°C, respiration is subsequently lowered (Table II). That is, respiration is lower even though actual determinations were made at 37.5° in phosphate saline. Similar effects can be obtained by changing the quality of serum. A greater percentage of serum is collected from blood if an animal is bled excessively. Simply stated, blood becomes more dilute as hemorrhage progresses, which is one of the physiological mechanisms for maintaining blood volume. As an example, serum B was obtained from a horse bled 8 liters. It was lighter in color and contained less solids than serum from a horse bled 4 liters (serum C). The latter serum appeared to contain more nutrients since it stimulated growth of cells better than the former. Metabolism was also higher for cells grown in the more concentrated serum (serum C) than for those grown in serum B as indicated in Table I. A similar effect appears to occur by lowering culture serum concentration from 20 to 10%, Fig. 2. Respiration was higher for cells grown in 20% serum than for those grown in 10%. Actually the values are not statistically different. The difference ($P<.08$) in respiration falls just below the arbitrary 5% level of significance and for this reason are considered indicative rather than factual.

Discussion. There appear to be 2 mechanisms which could explain the results in our experiments. Young or rapidly dividing cells have a metabolic rate different from that of old cells. Culture conditions which alter growth rate can also alter the ratio between young and old cells and therefore alter the metabolic picture. Our results and observations support this conclusion. Other evidence comes from an earlier report(6), in which we found that Strain L cells had a higher rate of oxygen uptake, if kept in a rapidly dividing state as compared to an old culture. Respiration of cultures fed the day before a Warburg test was twice that of cultures which had not been fed for 5 days.

When cells are transferred from one serum to another, aerobic lactate formation rises to a level which is out of proportion to oxygen uptake or anerobic lactate. Adaptation to the medium appears to occur in about 30

TABLE II. Effect of Growth Temperature or Serum on Respiration of Strain L Cells.

Serum in medium	Warburg solution	Growth temp.	Q_{O_2}	P
20%—C	Saline	37	3.3	
"	" + 10% serum A	"	3.5	
"	" + 10% " C	"	3.3	
20%—D	"	"	3.2	<.01
"	"	31	2.2	
20%—D	"	37	3.1	<.08
10%—D	"	"	2.8	

days and aerobic lactate returns to a moderate level. An explanation for this reaction or mechanism is unknown.

Our results introduce several important problems. If one wants to compare metabolism of one strain of tissue cultured cells with that of another they should be grown under the same conditions and in the same medium. The same applies if one compared metabolism of a normal tissue cultured cell to that of a tumor or virus infected one. Metabolic quotients for respiration or glycolysis of tissue cultured cells when considered alone, have little quantitative significance. These values cannot be duplicated precisely because sera from various laboratories will differ.

Although the exact nature of serum may not be known, various cell strains can be grown in a particular serum under identical conditions for a limited time, to compare metabolic rates. Cells derived from either normal or malignant tissue can thus be grown in tissue culture systems at a similar rate. Tissue cultured cell strains derived from normal or malignant tissue, in the same medium, growing at similar rate, have the same rate of respiration and lactate formation(1). Is it possible that respiration and glycolysis of a tumor differ basically from that of a normal tissue or could it be that the difference is due to a dominance of rapidly growing young cells? There is evidence that rapidly growing tissue such as bone marrow (11) and embryonic tissue, even when taken directly from the host, presents a metabolic picture similar to that associated with tumors.

Summary. Maximal growth of Earle's Strain L cells is obtained by using 15-20% horse serum with a medium containing 0.5% lactalbumin hydrolysate. Rate of lactate formation decreases as pH decreases but remains constant if the suspension is adequately buffered with bicarbonate. Respiration continues as a straight line function with time even though pH decreases. Sera from different horses affects growth rate of tissue cultures differently and as a consequence a culture in one horse serum may contain more rapidly dividing cells than another culture in another horse serum. Young, rapidly growing tissue cultured cell strains respire and glycolyze at a greater rate than old ones. This difference in metabolic rate occurs even though the cells are washed free of culture medium and resuspended in balanced saline solution before the metabolic determination is made. Metabolic rate is related to growth rate. This can be easily demonstrated by simply growing the cells at 31°C even though the actual determination is made at 37.5°C. Metabolic quotients obtained on tissue cultures grown in serum, have little quantitative significance when considered alone, because serum is not chemically definable.

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Received October 23, 1959. P.S.E.B.M., 1960, v103.