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Fetal Bovine Serum: A Multivariate Standard[1] (38804)

KENNETH V. HONN, JOHN A. SINGLEY, AND WALTER CHAVIN

Department of Biology, Wayne State University, Detroit, Michigan 48202

Progress in the attempt to provide a fully defined environment for cells and tissue in culture has been reviewed at intervals (1, 2). Nevertheless, it is still a widespread practice to correct deficiencies in and to add effectiveness to synthetic media with serum supplements (5–20%). The specific role and function of these supplements, however, remains questionable. As cell and organ culture approaches are utilized increasingly in quantitative experimental protocols, such serum supplements become important sources of variability.

Previous reports (3–5) dealing with microbial contamination in commercially available sera have revealed a variable degree of quality control. Additional variations in some chemical parameters of commercial fetal bovine sera have been reported (6–9). The increasing number of endocrine researches employing cell and organ culture techniques indicates the need for reevaluation of available sera in this respect. Since hormones are known to exert profound effects at concentrations in the picogram and nanogram range, and as these effects may be ubiquitous with regard to cell types, the presence of these chemoregulatory factors in serum-supplemented media has far-reaching implications. Thus, in the present study, routine chemical and hormone levels were determined in commercially available fetal bovine serum normally used as culture media supplements.

Materials and Methods. Fetal bovine serum was purchased from Hyland Laboratories, Grand Island Biological Company (Gibco) and Microbiological Associates (MBA). The sera were stored frozen (−20°C) and thawed (4°C) immediately prior to assay. The parameters determined on a Technicon Instruments Inc. SMA 12/60 autoanalyzer were: potassium, sodium, calcium, phosphate, chloride, uric acid, total bilirubin, urea (BUN), glucose, total protein, albumin, cholesterol, creatinine, alkaline phosphatase, creatinine phosphokinase (CPK), lactic dehydrogenase (LDH), and glutamic oxaloacetic transaminase (GOT). Osmolarity was determined by the freezing-point depression method. The hormones were assayed using commercially available radioimmunoassay (RIA) preparations (LH, FSH and testosterone, Serono Laboratories; TSH, Beckman Instruments; insulin, Pharmacia Laboratories; estradiol and thyroxine, Nuclear Medical Systems). In addition, commercially available antisera to cortisol (Endocrine Services) and ACTH (Burroughs Wellcome) were used for these hormones. The cortisol RIA followed the protocol of Endocrine Services. Serum was extracted (2×) with carbon tetrachloride (1:7, v/v) for corticosterone. The corticosterone RIA followed the protocol of Endocrine Services. The RIA for ACTH has been described previously (10). GH and prolactin content were assayed by RIA utilizing antisera to bovine GH and bovine prolactin. Total and pancreatic glucagon also were assayed by RIA.

Results and Discussion. The range of values for the serum parameters investigated (Table I) compared with those previously reported (6–9) demonstrate a poor correlation between independent investigations on separate lots of fetal bovine sera. Two investigations (6, 8) utilized specially processed sera in contrast to those commercially available which may account for some of the discrepancies. Nevertheless, a high degree of variability in a number of parameters is evident in the various lots of sera both between and within suppliers. Previously reported (6, 7) sodium values ranged widely. Only slight variations (106–112 meq/liter) in sodium content occurred between the lots of fetal bovine sera used in the present study. Chloride (130–163 meq/liter), calcium (9.4–15.5 meq/liter), and

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TABLE I. COMPARISON OF 32 PARAMETERS IN VARIOUS COMMERCIAL LOTS OF FETAL BOVINE SERUM

Profile parameters	Hyland	Gibco Lot A	Gibco Lot B	Gibco Lot C	MBA Lot A	MBA Lot B	MBA Lot C	Mean ± SD
Osmolarity (mosmol)	390	358	340	366	352	327.5	337	353 ± 20.8
K^+ [a]	23	11.9	6.6	5.6	11.8	1.8	7.2	9.7 ± 6.8
Na^+ [a]	111.2	112.3	106.1	111.0	102.5	109.7	105.9	108 ± 3.4
Cl^- [a]	163	147.6	134.7	149.3	137.4	130.1	136	142 ± 11.4
Ca^{+2} [a]	12.4	9.4	15.1	14.7	14.9	15.4	15.5	13.9 ± 2.2
PO_4^{-2} [a]	10.0	6.3	10.0	9.0	9.0	9.7	10.1	9.2 ± 1.3
Uric acid[b]	2.71	3.04	3.52	4.16	4.58	11.76	3.57	4.8 ± 3.2
BUN[b]	15.5	12.6	16.1	18.4	15.8	13.1	20.4	15.9 ± 2.7
Total bilirubin[b]	0.25	0.10	0.25	0.24	0.24	0.08	0.11	0.18 ± 0.08
Creatinine[b]	1.40	1.15	3.48	3.48	3.12	0.6	2.32	2.36 ± 1.25
Glucose[b]	353	382	322	353	316	256	249	318 ± 50
Cholesterol[b]	148	165	48	47	42	29	46	75 ± 56
Total protein[c]	7.97	8.49	3.79	3.87	3.81	3.04	3.70	4.95 ± 2.26
Albumin[c]	2.75	2.15	1.28	1.30	1.19	1.01	1.21	1.55 ± 0.63
ALK phosphatase[d]	140	37	147	175	205	138	177	146 ± 54
CPK[d]	417	283	118	164	99	77	152	187 ± 121
LDH[d]	598	598	460	615	71	132	588	437 ± 236
SGOT[d]	85	93	39	47	7	2	44	45 ± 35
T_4[e]	6.5	3.3	8.9	9.5	8.9	8.0	9.5	7.8 ± 2.2
Total cortisol[e]	12.5	14.0	7.2	7.7	9.3	9.7	7.0	9.6 ± 2.7
Free cortisol[e]	3.4	1.8	1.0	0.4	0.8	1.4	0.4	1.31 ± 1.04
Corticosterone[e]	2.5	0	0	0	0	0.1	0	0.37 ± 0.94
Testosterone[f]	80	35	47	56	79	32	45	53 ± 19
Insulin[g]	15.0	18.0	8.0	6.0	8.0	8.5	7.0	10.1 ± 4.5
Total glucagon[h]	730	845	80	185	70	20	70	286 ± 348
Pancreatic glucagon[h]	258	192	40	48	80	20	42	97 ± 91
ACTH[h]	11.0	48.0	6.0	11.0	11.0	11.0	6.0	14.9 ± 14.8
TSH[i]	1.0	<1.0	1.5	1.25	<1.0	<1.0	<1.0	1.1 ± 0.17
GH (bovine)[j]	70.8	23.2	114.9	140.3	88.6	4.1	167	87 ± 59
FSH[k, l]	1.56	1.56	1.56	1.56	1.56	1.56	1.56	1.56
LH[k]	4	2.5	1.5	1.5	1.0	1.0	1.0	1.78 ± 1.1
Prolactin (bovine)[j]	136	21.3	8.2	4.3	15.7	7.5	1.5	27.7 ± 48

[a] meq/liter.
[b] mg/dl.
[c] g/dl.
[d] mU/ml.
[e] μg/ml.
[f] ng/dl.
[g] μU/ml.
[h] pg/ml.
[i] μIU/ml.
[j] ng/ml.
[k] mIU/ml.
[l] Minimum detectable amount.

acid-soluble phosphorous (6.4–10.1 meq/liter) content varied more widely than sodium. Surprisingly, potassium content (1.8–23 meq/liter) was highly variable. In addition, chloride, potassium, and calcium values obtained in the present study demonstrated poor correlation with previously reported results (6, 7, 9). Serum osmolarity (328–390 mOsm/liter) which has not been previously reported, demonstrated considerable variability between lots examined.

Four metabolites were evaluated in terms of their variability and the possibility that toxic levels to cells may be present in serum-supplemented media. Analysis of uric acid, urea, total bilirubin, and creatinine levels indicated that a 2- to 6-fold variation was present. The greatest variation occurred in uric acid (2.71–11.8 mg/dl) and creatine (0.6–3.5 mg/dl) levels with total bilirubin (0.08–0.25 mg/dl), and urea (12.6–20.4 mg/dl) levels being somewhat more consistent.

The importance of cholesterol as an attachment factor for increased plating efficiency has been reported (11). However, with the varied levels of cholesterol (29–165 mg/dl) in different lots of fetal bovine sera, reliance upon serum supplements for consistent plating efficiency is tenuous. Glucose as an energy source is included in most chemically defined media. Additional glucose in the form of serum supplements were considerable and varied (249–382 mg/dl) perhaps depending on the nutritional history of the animal and glycolytic and gluconeogenic hormone levels at the time of sacrifice. Serum protein content increases with the age of the fetus (6); therefore, the observed range protein values (3.04–8.49 g/dl) was not surprising. Albumin (1.01–2.75 g/dl) levels were not as varied and were similar to those previously reported (6, 9).

Lactic dehydrogenase (71–615 mU/ml), creatinine phosphokinase (77–417 mU/ml), alkaline phosphatase (37–205 mU/ml) and glutamicoxaloacetic transaminase (2–93 mU/ml) activities were extremely variable and often elevated in the sera examined. In addition, aspartate aminotransferase has been reported (7) in fetal bovine serum. The effect of such serum enzymes upon cells in culture has not been determined. As these constituents, unlike potassium, glucose, and urea, cannot be removed by molecular sieve treatment or dialysis (9), consideration of these elevated concentrations and variability is merited.

Varied biological effects of serum supplements upon cultured cells have been reported; for example, final population density (11), DNA synthesis (12), protein synthesis (11), mitosis (13), and cloning efficiency (13). Attempts to isolate the serum factors responsible for these biological effects have delineated many possibilities (14–16). However, consideration must be given to the chemoregulatory agents, although a qualitative and quantitative survey of commercial fetal bovine sera in this regard has not been reported. Considerable *in vivo* variability of growth hormone, LH, prolactin and T_4 has been noted in bovine fetuses of different sex and stages of gestation (17, 18). It was not unreasonable, therefore, to expect similar variation between different lots of fetal bovine sera. Serum T_4 levels (3.3–9.5 μg/dl) reflect the variations expected between 90 and 180 days of gestation (17). Similarly GH (4.1–167 ng/ml) and prolactin (1.5–136 ng/ml) demonstrated considerable variation while the gonadotropins FSH (1.56 mIU/ml) and LH (1–4 mIU/ml) levels remained low. Considering the other pituitary polypeptides, TSH (1–1.5 μIU/ml) levels were consistent while ACTH (6–48 pg/ml) levels were widely varied. Examination of steroid hormone levels revealed that testosterone (32–80 ng/dl) levels fluctuated widely. Total (7–14 μg/dl) and free (0.4–3.4 μg/dl) cortisol levels presented 2- and 8-fold variations, respectively, while corticosterone (0–2.5 μg/dl) was undetectable in five of the serum lots examined. Extreme variations were present in total (20–845 pg/ml) and pancreatic (20–258 pg/ml) glucagon. Insulin concentrations (6–18 μU/ml) varied 3-fold.

With the ultimate goal of eliminating both the unknown and the considerable variability of serum supplements, attempts have been made to develop serum substitutes (19). However, at present, such substitutes offer limited applicability. Consequently a surveillance procedure applied to the production of fetal bovine sera has been suggested (8) but parameters surveyed are limited. Further, dialysis or Sephadex G-50 treatment of sera

is reported (9) to reduce serum constituents thereby eliminating several factors (potassium, glucose, and urea), but other factors (serum enzymes, hormones) are not eliminated from consideration by this treatment.

Until the development of adequate serum substitutes, the present findings suggest that investigations utilizing culture techniques dependent upon serum supplements be preceded by a screening procedure to determine the levels of any serum components which have antagonistic or synergistic effects on test substances and/or cell types under investigation.

Summary. Chemical and endocrine parameters were investigated in commercially available fetal bovine sera intended for use as culture media supplements. A high degree of serum variability was present both within and between suppliers in all major categories investigated. It is suggested that caution be employed in the interpretation of results from experiments utilizing serum supplements without specific quantitation of possible interfering or modulating factors.

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