kidneys from rats rendered hypoxic for 18 hours. This factor has been purified approximately 230-fold by ammonium sulfate precipitation, DEAE–cellulose column chromatography and Sephadex gel filtration.

1. Contrera, J. F. and Gordon, A. S., Science 152, 653 (1966).
2. Zanjani, E. D., Cooper, G. W., Gordon, A. S., Wong K. K., and Scribner, V. A., Proc. Soc. Exptl. Biol. Med. 126, 540 (1967).
3. Zanjani, E. D., Contrera, J. F., Cooper, G. W., Gordon, A. S., and Wong, K. K., Science 156, 1367 (1967).
4. Zanjani, E. D., Contrera, J. F., Gordon, A. S., Cooper, G. W., Wong, K. K., and Katz, R., Proc. Soc. Exptl. Biol. Med. 125, 505 (1967).
5. Gordon, A. S., Cooper, G. W., and Zanjani, E. D., in "Seminars in Hematology" Miescher, P. A., ed., Vol. 4, p337. Grune and Stratton, New York, 1967.
6. LaBella, F. S., Reiffenstein, R. J., and Beaulieu, G., Arch. Biochem. Biophys. 100, 399 (1963).
7. Gornall, A. G., Bandawill, C. S., and Davis, M. M., J. Biol. Chem. 177, 751 (1949).
8. Lowry, D. H., Rosenbrough, N. J., Farr, L., and Randall, R. J., J. Biol. Chem. 193, 265 (1951).
9. Weintraub, A. H., Gordon, A. S., and Camiscoli, J. F., J. Lab. Clin. Med. 62, 743 (1963).

Received Jan. 2, 1968. P.S.E.B.M., 1968, Vol. 128.

Plasma Growth Hormone in the Infant Undergoing Deep Hypothermic Cardiovascular Surgery*† (32945)

DAVID BAUM, CHARLES C. GALE, AND DAVID H. DILLARD
(Introduced by Daniel Porte, Jr.)
Departments of Pediatrics, Physiology and Biophysics, and Surgery, University of Washington School of Medicine, Seattle, Washington 98105

Growth homone in man is involved in fuel metabolism as well as in the promotion of growth (4). Both its stimulatory effect on lipolysis (11) and its anti-insulin effect (4) relate it to lipid and carbohydrate pathways. The effect of growth hormone on free fatty acid mobilization and on insulin suggests that it may have a metabolic role in alterations of heat production. Therefore, the release of growth hormone has potential physiologic importance in hypothermic man.

Utilization of plasma glucose is diminished at low body temperatures in several mammals (23,18), including man (1,6,18). In many situations associated with decreased glucose utilization, the release of growth hormone is increased, as with the administration of 2-deoxy-D-glucose (14) and in diabetic patients with severe ketoacidosis (16). Furthermore, cold stress itself has been found to stimulate the release of growth hormone in nonprimate species (8,9). These observations suggesting increased release of growth hormone under conditions of low body temperature prompted an investigation of plasma growth hormone in patients undergoing deep hypothermic cardiovascular surgery.

This investigation represents a departure from earlier studies (8,9,14,16) in that the subjects were young infants and each was cooled to a body temperature below 20°C. Since hypothermia was surface induced, all had intact cardiovascular systems during cooling, eliminating any effect that might have been contributed by the use of cardiopulmonary bypass.

Methods. Six infants, age 1.5 to 16 months, who underwent cardiovascular surgery were studied (Table I). Corrective heart surgery was performed in all except L.G., who had a palliative operation. Body temperature was lowered to 15–20°C by surface cooling. Hyperventilation with high concentrations of oxygen was used to induce respiratory alkalosis during cooling and rewarming in order to offset the development of metabolic acidosis (10). Deep ether anesthesia was used from

* Supported by a Grant from the Montana Heart Association.

† Presented in part at the International Symposium on Growth Hormone, Milan, Italy, September 13–15, 1967.

TABLE I. List of Patients.

Patient	Age (months)	Cardiac abnormality	Outcome
M.P.	13	VSD	Survived
T.D.D.	1.5	Transposition of great vessels	Survived
L.G.	3	A–V canal, pulmonary atresia, PDA	Died
C.S.	3	Transposition of great vessels	Survived
J.F.	13	Total anomalous pulmonary venous return	Survived
T.R.	16	Double outlet right ventricle	Died

the start to the procedure through early cooling, and occasionally for a short period during rewarming. Cardiac arrest was induced by cross clamping the aorta and coronary injection of potassium ion, and cardiac repair accomplished when core temperature fell below 20°C. An exception was patient L.G., in whom a left subclavian-pulmonary artery anastomosis (Blalock-Taussing Procedure) was performed while her circulation was allowed to continue. Following cardiac repair, core temperature was returned to the euthermic range by surface warming. Supraventricular cardiac rhythms were present throughout cooling, returning with cardiac resuscitation after circulatory arrest.

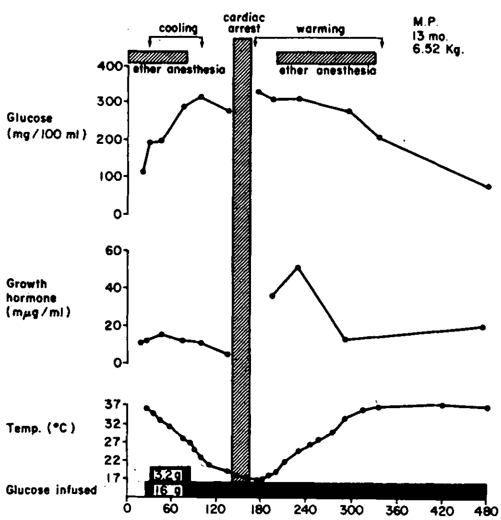

FIG. 1. Glucose infusion: Plasma growth hormone levels mildly increased with anesthesia, changed little with cooling, rose markedly during early rewarming, and returned to baseline concentrations as core euthermia was achieved. Pronounced hyperglycemia was associated with hypothermia. Note that both plasma growth hormone and glucose changes occur despite the presence of ether anesthasia.

Continuous glucose infusions were administered to four patients (Table II) after baseline samples were obtained on ether anesthesia alone. The rates of infusion were roughly constant, although the rate differed for each patient (0.16–0.5 gm/kg per hour). Blood loss in all patients was replaced with A–C–D[1] bank blood, but in most cases this was not required before core temperature reached a range of 20°C. The bank blood contained glucose (300–350 mg/100 ml) and growth hormone (<3 mμg/ml). Two patients did not receive glucose except for that in the replacement transfusions resulting in additions of glucose which were less than 0.05 gm/kg per hour.

All blood samples were obtained from a peripheral artery. Plasma glucose was determined by the glucose oxidase method (7) and growth hormone was measured by a modification of the double antibody immunoprecipitation technique (15). Deep rectal temperature was recorded with a Yellow Springs Instrument Company telethermometer and appropriate probes.

Results. Glucose. Baseline plasma glucose concentrations drawn during anesthesia ranged between 80–153 mg/100 ml (Table II). In those infants given glucose infusions, precooling elevations of plasma glucose were less than 90 mg/100 ml. Although the rates of infusion remained unchanged, further increases in plasma glucose developed with cooling as body temperature fell below 30–35°C. The effect of hypothermia on plasma glucose during glucose infusion is illustrated by M.P. (Fig. 1). The added increases in plasma glucose concentration with deep hypothermia ranged between 140–360 mg/100 ml. Marked hyperglycemia persisted during

[1] acid–citrate–dextrose.

TABLE II. List of Data.

Patient	Time from onset (min)	Temperature (°C)	Glucose[a] (mg/100 ml)	Growth hormone (mμg/ml)
A. Glucose infused				
M.P.	20	36.5	114	11
	25	36.0	192	12
Glucose infused 0.5 gm/kg per hour	45	32.8	198	15
	75	28.0	288	12
	98	22.7	316	11
	136	19.5	279	11
	166	17.2	330	5
	195	19.0	307	36
	230	25.0	309	51
	295	35.0	279	13
	335	37.0	206	—
	480	37.3	88	20
T.D.D.	68	31.8	129	23
	85	31.0	131	17
Glucose infused 0.16 gm/kg per hour	105	31.0	151	15
	145	30.0	182	12
	165	25.6	229	9
	180	24.0	287	10
	215	19.9	278	10
	287	19.8	307	47
	320	19.3	311	61
	358	28.5	293	51
	388	31.9	320	57
	423	35.0	231	53
	493	36.2	120	19
L.G.	85	35.7	96	24
	105	34.7	143	25
Glucose infused 0.5 gm/kg per hour	125	31.0	165	21
	143	26.4	191	15
	152	24.5	210	16
	183	20.0	118	15
	215	19.0	500	10
	247	20.5	322	8
	285	25.0	422	—
	335	30.0	342	30
	360	33.0	310	22
	397	36.0	398	101
C.S.	20	37.1	82	—
	50	36.9	360	75
Glucose infused 0.5 gm/kg per hour	60	36.8	250	97
	70	35.5	193	151
	95	35.0	174	—
	105	34.0	260	143
	125	30.0	295	109
	155	25.3	365	77
	180	21.0	425	60
	188	20.0	335	52
	200	19.0	410	52

TABLE II (continued)

Patient	Time from onset (min)	Temperature (°C)	Glucose[a] (mg/100 ml)	Growth hormone (mμg/ml)
	218	18.0	355	55
	244	16.8	410	62
	310	18.5	422	89
	340	27.4	535	83
	390	32.2	503	151
	430	34.8	482	133
	462	36.4	470	113
	498	37.3	432	57
B. No glucose infused				
J.F.	113	38.0	153	23
	125	38.0	149	22
	134	38.0	175	29
	150	37.0	155	28
	165	35.0	153	31
	195	29.0	201	27
	215	25.5	232	20
	258	22.6	210	16
	305	19.9	211	16
	350	22.0	195	14
	387	24.3	205	24
	435	29.3	241	28
	490	35.1	226	25
	540	37.0	115	11
	630	37.2	134	10
T.R.	47	37.4	80	21
	62	37.4	78	19
	105	33.2	84	17
	137	25.0	92	16
	168	20.0	95	15
	203	19.0	93	11
	278	16.8	162	—
	302	17.9	129	—
	327	21.8	111	90
	370	28.8	111	—
	405	31.8	66	123

[a] Glucose data has been reported in part (1).

warming until the infants' body temperature was raised above 30–35°C when a regression of the hyperglycemia occurred.[2]

In the two patients not receiving glucose infusions, plasma glucose was not elevated above precooling levels despite the development of hypothermia (Table II). The stable courses of plasma glucose were maintained in both these patients until replacement transfusion with hyperglycemic bank blood was begun. This stability is shown by J.F. (Fig. 2). The modest elevations of plasma glucose then occurring in both these patients (90 mg/100 ml in J.F. and 80 mg/100 ml in T.R.) regressed with rewarming.

Growth hormone. Baseline plasma levels of growth hormone obtained while on ether anesthesia ranged between 10 and 24 mμg/ml in all patients except C.S. This latter patient developed a peak growth hormone concentra-

[2] This regression did not occur in L. G., who received epinephrine during late rewarming.

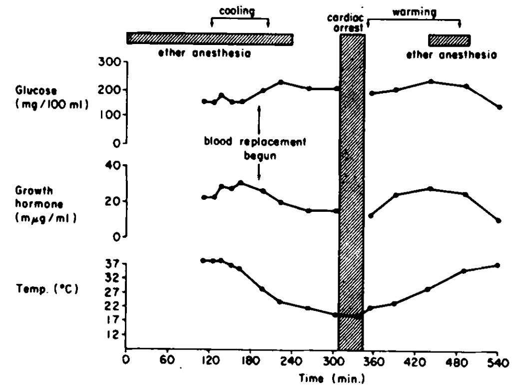

FIG. 2. No glucose infusion: Plasma growth hormone levels, high with anesthesia, fell slightly with cooling and returned to baseline levels with rewarming. Plasma glucose concentrations remained relatively stable with cooling until transfusion with hyperglycemic blood was initiated. Patient J. F., 13 months, 6.5 kg, no glucose infused.

tion of 151 mμg/ml with an episode of hypoxia during the induction of anesthesia. With cooling, the concentrations of growth hormone in all patients fell, or remained essentially unchanged. On rewarming, the concentration of growth hormone rose, often rising above precooling levels. Peak values obtained with rewarming were between 29 and 151 mμg/ml. In some, these peak values were achieved early in rewarming, while body temperature was still low. In two patients, the highest levels were found at the end of the procedure. These two patients represent the two operative deaths in the group. Both died shortly after the last sample was obtained. In summary, the course of plasma growth hormone was similar for all patients whether or not glucose infusions were administered (Figs. 1 and 2). Further, there was no apparent relationship between levels of plasma glucose and growth hormone (Table II).

Discussion. The high (17) baseline plasma glucose concentrations were probably the result of catecholamine release with ether anesthesia (12). However, inhibited utilization of plasma glucose appears to have been the predominant factor producing the inordinate hyperglycemia found during hypothermia. This conclusion is based upon two observations in our patients: (i) the inordinate hyperglycemia was found only in infants given glucose infusions, and (ii) in the infants not receiving glucose infusions, elevation above prehypothermic plasma glucose concentrations did not occur until transfusion with hyperglycemic blood was begun. The work of others (2,3,6,18) supports this interpretation of our data.

Baseline levels of growth hormone obtained during ether anesthesia were above those found in resting infants of comparable ages (5). These findings are consistent with the observations of Glick *et al.* (5) who demonstrated increased levels of growth hormone during anesthesia. With body cooling, plasma levels of growth hormone did not increase further, perhaps due to the inability of the hypothalamic–pituitary axis to respond to this additional stress. Alternatively, it may be that hypothermia is not a specific stimulus for growth hormone release in the infant as it appears to be for certain nonprimate mammals (8,9). In fact, review of the present data suggests no specific relation between plasma levels of growth hormone and either body temperature or plasma glucose. Furthermore, plasma growth hormone levels were not consistently related to inferred changes in glucose utilization. The lack of relationship between plasma growth hormone levels on one hand and body temperature, plasma glucose levels, or glucose utilization on the other implies that other factors may have influenced the release of growth hormone in the infants studied. Our investigation of growth hormone involved operated infants while earlier reports of the effects of diminished glucose utilization and cold stress on circulating growth hormone were performed in nonprimates and nonoperated adults. The disparity between the present findings and those of previous investigators suggests the possibility of a species or age difference, or an effect of surgical intervention. In any case, these data indicate that the mechanism for growth hormone release in the operated hypothermic infant is incompletely understood. The present data, however, suggest that growth hormone may play a role in recovery from hypothermic surgery in man. It is possible that growth hormone in this situation serves more importantly via its anticatabolic action

(13) to spare essential protein tissues rather than to mobilize substrate.

Summary and Conclusions. A study of plasma growth hormone and glucose, and body temperature, was conducted in six infants undergoing deep hypothermic cardiovascular surgery. Pronounced hyperglycemia occurred during hypothermia when the infants received exogenous glucose, confirming earlier reports of diminished plasma glucose utilization at low body temperatures. Although growth hormone levels were high during ether anesthesia, they fell or changed little with cooling, and did not appear related to core temperature or to plasma glucose concentration. These findings indicate that changes in growth hormone levels in operated hypothermic infants did not relate to cold stress, alterations in plasma glucose concentration, or diminished glucose utilization.

1. Baum, D., Dillard, D. H., Mohri, H., and Crawford, E. W., Pediatrics, in press.

2. Bickford, A. F. and Mottram, R. F., Clin. Sci. 19, 345 (1960).

3. Blackard, W. G., Nelson, N. C., and Labat, J. A., Am. J. Physiol. 212, 1185 (1967).

4. Daughaday, W. H. and Kipnis, D. M., Recent Progr. Hormone Res. 22, 49 (1966).

5. Glick, S. M., Roth, J., Yalow, R. S., and Berson, S. A., Recent Progr. Hormone Res. 21, 241 (1965).

6. Henneman, D. H., Bunker, J. P., and Brewster, W. R., Jr., J. Appl. Physiol. 12, 164 (1958).

7. Huggett, A. St. G. and Nixon, D. A., Biochem. J. 66, 12P (1957).

8. Krulich, L. and McCann, S. M., Proc. Soc. Exptl. Biol. Med. 122, 612 (1966).

9. Machlin, L. J., Takahashi, Y., Horino, M., Hertelendy, F., Gordon, R. S., and Kipnis, D. M., Intern. Symp. Growth Hormone, Excerpta Medica Intern. Congr. Ser. 1967, No. 142. Abstract No. 26.

10. Mohri, H., Hessel, E. A., Jr., Nelson, R. J., Matano, I., Anderson, H. N., Dillard, D. H., and Merendino, K. A., Am. J. Surg. 112, 241 (1966).

11. Raben, M. S. and Hollenberg, C. H., J. Clin. Invest. 38, 484 (1959).

12. Richardson, J. A., Woods, E. F., and Richardson, A. K., J. Pharmacol. Exptl. Therap. 119, 378 (1957).

13. Root, A., Pediatrics 36, 940 (1965).

14. Roth, J., Glick, S. M., Yalow, R. S., and Berson, S. A., Metab. Clin. Exptl. 12, 577 (1963).

15. Schalch, D. S. and Parker, M. L., Nature 203, 1141 (1964).

16. Unger, R. H., J. Am. Med. Assoc. 191, 149 (1965).

17. Wolman, I., "Laboratory Applications in Clinical Pediatrics," p.498. McGraw-Hill, New York 1957.

18. Wynn, V., Lancet 2, 575 (1954).

Received Jan. 8, 1968. P.S.E.B.M., 1968, Vol. 128.

Fractionation of an Esterase from Calf Spleen Implicated in the Detoxification of Bacterial Endotoxin* (32946)

ROBERT SKARNES,[1] SELMA RUTENBURG, AND JACOB FINE
Department of Surgery, Beth Israel Hospital, Harvard Medical School, Boston, Massachusetts

The rapid uptake and detoxification of bacterial endotoxin by dog spleen *in situ* has been effectively demonstrated (1,2). Furthermore, crude extracts of splenic tissue have been shown to possess a strong detoxifying action against endotoxins *in vitro* (2,3). The present investigation was prompted by the recent finding that nonspecific carboxylic esterases from normal serum were implicated in the degradation and inactivation of endotoxin (4). The fractionation of calf spleen extracts and the identification of an esterase apparently responsible for the detoxification of endotoxin are described.

Materials and Methods. Whole calf spleens were removed from exsanguinated animals and either frozen immediately or carefully perfused with saline before freezing. Subsequently, frozen spleens were quickly thawed

* Supported by a grant from Merck and Co., Rahway, New Jersey.

[1] Present address: Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545.