than SV_{20}. The data obtained in hemagglutination inhibition tests with SV_{20} virus hyperimmune rabbit serum make it appear, however, that SV_{20} is distinct from any of the 28 adenoviruses of human origin against which it was tested. It might be profitable to look for a primary infection occurring commonly in children in New Guinea and less frequently in children in the United States which is accompanied by development of SV_{20} virus neutralizing antibody. In addition, search might also be made of a recurrent infection occurring in adults in presence of SV_{20} antibody.

Summary. Sera from 267 infants, children, and adults living in 2 markedly different ecologic settings, New Guinea and the United States, were examined for presence of neutralizing antibodies to monkey myxovirus SV_5 and monkey adenovirus SV_{20}. SV_{20} neutralizing antibody in titers of 1:10 to 1:160 were present in New Guinea in 70% of ½- to 5-year olds; in 72% of 6- to 18-year olds; and in 78% of adults. This antibody was absent in the U. S. in individuals in the ½- to 5-year old age group, but was present in titers of 1:10 to 1:80 in 33% of 6- to 18-year olds and in 22% of adults. None of the New Guinea sera and only 8% of the U. S. sera possessed SV_5 neutralizing antibody. These data suggest the possibility that SV_5 and SV_{20}, or agents antigenically related to them, might be infectious for man.

1. Hull, R. N., Minner, J. R., *Ann. N. Y. Acad. Sci.*, 1957, v67, 413.
2. Hull, R. N., Minner, J. R., Mascoli, C. C., *Am. J. Hyg.*, 1958, v68, 31.
3. Malherbe, H., Harwin, R., *Brit. J. Exp. Path.*, 1957, v38, 539.
4. Sabin, A. B., Wright, A. M., *J. Exp. Med.*, 1934, v59, 115.
5. Sweet, B. H., Hilleman, M. R., PROC. SOC. EXP. BIOL. AND MED., 1960, v105, 420.
6. Morris, J. A., Johnson, K. M., Aulisio, C. G., Chanock, R. M., Knight, V., *ibid.*, 1961, v108, 56.
7. Morris, J. A., Blount, R. E., Jr., Savage, R. E., *ibid.*, 1956, v92, 544.
8. Chanock, R., Roizman, B., Myers, R., *Am. J. Hyg.*, 1957, v66, 281.
9. Rosen, L., *ibid.*, 1960, v71, 120.
10. Gold, E., Ginsberg, H. S., *J. Immunol.*, 1962, v88, 513.
11. Takemori, N., Nomura, S., Nakano, M., Morioka, Y., Henmi, M., Kitaoka, M., *Virology*, 1958, v5, 30.
12. Morris, J. A., O'Connor, J. R., *Cornell Vet.*, 1952, v42, 56.
13. Chanock, R. M., Johnson, K. M., Cook, M. K., Wong, D. C., Vargosko, A., *Am. Rev. Resp. Dis.*, 1961, v83, 125.
14. Hsiung, G. D., Isacson, P., McCollum, R. W., *J. Immunol.*, 1962, v88, 284.
15. Schultz, E. W., Habel, K., *ibid.*, 1959, v82, 274.
16. Jensen, K. E., *Am. Rev. Resp. Dis.*, 1961, v83, 120.
17. Stone, J. D., *Australian J. Exp. Biol. Med. Sci.*, 1948, v26, 49.

Received May 22, 1964. P.S.E.B.M., 1964, v117.

Effect of Glucose Infusion on the Individual Plasma Free Amino Acids in Man.* (29483)

O. B. CROFFORD,† P. W. FELTS‡ AND W. W. LACY§
(Introduced by Elliot V. Newman)
Department of Medicine, Laboratories of Clinical Physiology, Vanderbilt University, Nashville, Tenn.

There is a recognized relationship between the concentration of plasma free amino acids

* Supported by U. S. Public Health Service grants AM-04361-03(MET) and HE-04720-04(MET).

† Performed in part during the tenure of a Postdoctoral Fellowship, United States Public Health Service. Present address: Institut de Biochimie Clinique, Université de Genéve, Sentier de la Roseraie, Geneva, Switzerland.

‡ Fellow of the Life Insurance Medical Research Fund. Present address: II Medizinische Klinik, der Universität Munchen, Zienssenstrasse, Munich, Germany.

§ Established Investigator, American Heart Association.

and a sudden change in concentration of blood glucose. Albanese and his co-workers showed the depressing effect of oral glucose on the blood amino nitrogen(1). Munro, measuring 7 of the individual amino acids, reported this effect to be specific for the essential amino acids(2).

This study reports the plasma concentration of 22 individual amino acids measured before and 60 minutes following a rapid infusion of glucose, with and without exogenous insulin, in 12 healthy men. To determine which of the individual amino acids were most responsive to this stimulus, the glucose infusion was regulated to yield a submaximal depression of the total plasma free amino acids. The plasma free fatty acid (FFA) and total free amino acid (FAA) responses indicated the degree to which the overall metabolic energetics of the subjects were affected by the glucose infusion, and the magnitude of the insulin effect.

Materials and methods. The subjects consisted of 12 healthy males between the ages of 20 and 31, weighing between 128 and 265 lb. All tests were performed at 8:00 AM following a 14-hour fast. An indwelling sampling needle was placed in the antecubital vein of the right arm and a standard infusion needle in the antecubital vein of the opposite arm. After obtaining the control blood samples, an infusion of 10% glucose was begun with each subject receiving 25 g of glucose over a 5-minute period. When insulin was given, 0.1 unit per kg body weight was added to the glucose infusion.‖ Control studies were performed by infusing the subjects with 0.9% sodium chloride in place of glucose. Fifty ml of venous blood were obtained before and 60 minutes after the glucose infusion for the individual amino acid analyses. Ten ml of blood were obtained before the glucose infusion and at 5-minute intervals for a period of 60 minutes for analyses of glucose, FFA and total FAA. All samples were anticoagulated with powdered heparin and the plasmas collected by centrifugation at 4°C. The plasmas for the individual amino acid analyses were subjected to ultrafiltration under 400 psi nitrogen. The ultrafiltrate was acidified and stored at —20°C until analyzed. The individual amino acids were determined by column chromatography(3) using a Phoenix Precision Instrument K5000. Plasma glucose concentration was determined by the glucose oxidase-catalase method of Sunderman and Sunderman (4). Plasma FFA concentration was determined by a modification of the Dole method (5). Plasma FAA concentration was determined by a modification(6) of the method of Van Slyke(7).

‖ Crystalline Zinc Insulin, Lilly, Glucogon-free Lot #W-3603 was generously provided by Dr. K. K. Chen, Eli Lilly Co.

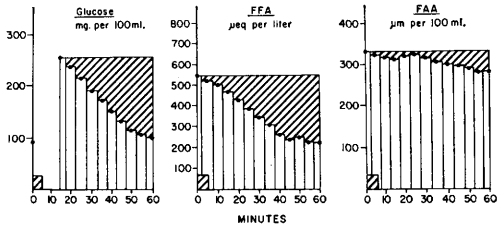

FIG. 1. Plasma glucose, FFA and total FAA concentration changes. The 5 minute glucose infusion is represented by cross hatched area in lower left hand corner of each figure. Plasma glucose concentration was not measured for the 10 minutes immediately following glucose infusion to allow for complete mixing. Concentration changes are expressed as the ratio of the cross hatched area in upper right hand corner of each rectangle to total rectangular area. A value approaching 1.0 indicates a maximum change while a value near 0 indicates a minimum change. This method was used so that the fall in plasma FFA concentration (which could not be approximated by an exponential curve) could be compared to the fall in plasma glucose concentration. Values in Table I were derived in this manner.

Results and discussion. The results of a representative experiment are shown in Fig. 1, with the data from 18 such experiments summarized in Tables I and II. Table I shows that the stimulus of the glucose infusion was sufficient to cause a significant fall in plasma FFA concentration and that this effect was enhanced by addition of exogenous insulin. As expected(8) the rate and magnitude of the fall in plasma FFA concentration was proportional to the rapidity with which the blood glucose concentration returned toward the pre-infusion level. The fall in plasma total FAA was not significant in the absence of exogenous insulin. Even in

TABLE I. Simultaneous Changes in Plasma Concentration of Glucose, FFA and Total FAA.

Infusion	Glucose	FFA	Total FAA
Glucose (10)	.272 ± .011[a]	.310 ± .022[a]	.038 ± .012[c]
" + insulin (6)	.564 ± .020[b]	.551 ± .024[b]	.060 ± .014[c]
Saline (2)	.005	.006	.024

Infusions were given as described in text. Values were derived as explained in legend of Fig. 1. Numbers in each column represent mean ± 1 standard error of mean. Number of observations in parentheses. Significance: [a] p *vs* saline control <.001, [b] p *vs* glucose <.001, [c] not significantly different from appropriate control.

TABLE II. Changes in Plasma Concentration of Amino Acids in Response to Infusions of Glucose and Glucose + Insulin in Normal Men.

	Glucose			Glucose + Insulin		
Amino acid	Pre-infusion	Post-infusion	p	Pre-infusion	Post-infusion	p
Glutamine & asp.	(6) 59.1 ± 3.8	56.5 ± 5.5	NS	(3) 53.3 ± 2.4	37.1 ± 4.0	.025
Alanine	(6) 41.6 ± 5.0	38.8 ± .6	NS	(3) 29.3 ± 5.6	21.8 ± 6.8	NS
Glycine	(6) 25.9 ± 4.5	23.5 ± 2.8	NS	(3) 20.4 ± 3.1	17.0 ± 3.9	NS
Valine	(6) 24.2 ± 2.3	19.2 ± 1.4	.005	(3) 18.6 ± 2.6	13.5 ± 2.5	.05
Proline	(6) 23.5 ± 6.0	20.8 ± 4.4	NS	(3) 22.9 ± 1.7	15.6 ± 1.7	.025
Lysine	(4) 16.5 ± 2.6	16.3 ± 3.7	NS	(3) 12.5 ± 1.9	11.9 ± 2.6	NS
Threonine	(6) 15.3 ± 1.6	12.9 ± 1.6	.05	(3) 11.4 ± .7	8.1 ± 1.5	.05
Serine	(6) 12.2 ± 2.0	10.2 ± 2.5	NS	(3) 9.9 ± .2	6.9 ± 1.5	.05
Leucine	(6) 12.6 ± 1.0	9.4 ± .2	.005	(3) 11.0 ± 1.9	6.3 ± 1.6	.025
Arginine	(3) 8.3 ± .7	7.3 ± 3.6	NS	(3) 6.6 ± 1.2	5.7 ± 1.6	NS
Isoleucine	(6) 7.8 ± 1.3	5.6 ± 1.2	.01	(3) 6.2 ± .8	3.3 ± .8	.05
Histidine	(4) 6.8 ± 3.3	7.9 ± 5.0	NS	(3) 5.5 ± 1.8	6.5 ± 1.6	NS
Cystine	(6) 6.1 ± 1.1	5.6 ± 1.0	NS	(3) 4.6 ± .7	3.0 ± 1.7	.10
Phenylalanine	(6) 5.3 ± .2	4.2 ± .02	.025	(3) 4.5 ± 1.2	3.4 ± 1.1	NS
Tyrosine	(6) 4.9 ± 1.0	4.2 ± .12	.05	(3) 5.3 ± .7	3.6 ± .9	.10
Taurine	(6) 4.5 ± 1.2	4.7 ± .5	NS	(3) 3.8 ± 1.3	3.4 ± .9	NS
Ornithine	(4) 4.5 ± 1.9	5.2 ± 1.6	NS	(3) 4.8 ± .5	4.3 ± .9	NS
Glutamic acid	(6) 3.6 ± 2.3	2.8 ± 1.0	NS	(3) 3.3 ± .8	3.3 ± 1.4	NS
Citrulline	(6) 3.6 ± .7	3.6 ± .93	NS	(3) 3.1 ± .5	2.0 ± .6	.10
Methionine	(6) 2.8 ± .9	2.5 ± .2	NS	(3) 2.5 ± .7	1.5 ± .7	NS
Amino-n-butyric	(6) 2.5 ± .8	2.6 ± .7	NS	(3) 1.8 ± .2	1.4 ± .1	.10
Total	291.6 ± 7.8	263.8 ±10.4		241.3 ±24.2	179.6 ±38.4	

Plasma concentration of amino acids is expressed as μmoles per 100 ml. Values given are mean and 1 standard deviation from mean. No. of observations in parentheses. p = probability that the difference between pre-infusion and post-infusion means equals zero. NS = not significant.

the presence of exogenous insulin, the fall in plasma total FAA was less than the values reported by Munro(2) and by Rubini(9) who showed 60 minute reductions of 12 and 14% respectively.

In Table II, the amino acids which were most responsive to the stimulus of the glucose infusion were valine, leucine, isoleucine and threonine-phenylalanine, in that order. These findings were in general agreement with those of Munro(2) in the 7 individual amino acids which they measured. Glycine and alanine, though occasionally showing a fall, frequently rose in concentration. Although no marked changes in the pattern of amino acid fall occurred with the addition of insulin, the effect on most of the amino acids appeared to be enhanced.

Summary. Glucose, with and without exogenous insulin, was infused into normal males under conditions which produced a submaximal fall in total plasma FAA concentrations. Analysis of the individual free plasma amino acids revealed a significant decrease in the following essential amino acids; leucine, valine, threonine, isoleucine, phenylalanine and tyrosine. This effect is enhanced by the presence of exogenous insulin.

1. Albanese, A. A., Orto, L., Rossy, J., DiLallo, R., Belmont, A., *Metabolism,* 1955, v4, 160.

2. Munro, H. N., Thompson, W. S. T., *ibid.,* 1953, v2, 354.

3. Spackman, D. H., Stein, W. H., Moore, S., *Anal. Chem.,* 1958, v30, 1190.

4. Sunderman, F. W., Sunderman, F. W., Jr., *Measurements of Exocrine and Endocrine Functions of the Pancreas,* Lippincott, Philadelphia, 1961, 128.
5. Dole, V. P., *J. Clin. Invest.*, 1956, v35, 150.
6. Lacy, W. W., Crofford, O. B., submitted for publication, *J. Lab. and Clin. Med.*
7. Hamilton, P. B., Van Slyke, D. D., *J. Biol. Chem.*, 1943, v150, 231.
8. Bierman, E. L., Schwartz, I. L., Dole, V. P., *Am. J. Physiol.*, 1957, v191, 359.
9. Rubini, M. E., Seligson, D., *Am. J. Clin. Nutr.*, 1958, v6, 365.

Received May 26, 1964. P.S.E.B.M., 1964, v117.

Fever and Release of Endogenous Pyrogen in Leukopenic Rabbits.* (29484)

JONAS A. SHULMAN AND ROBERT G. PETERSDORF
Department of Medicine, University of Washington School of Medicine and King County Hospital, Seattle

Following administration of bacterial endotoxins, rabbits develop profound leukopenia followed by fever and leukocytosis. During the period of leukopenia, the granulocytes are presumably injured by the toxin and release a humoral substance, endogenous pyrogen (EP), which acts directly on the thermoregulatory centers to produce fever(1). Support for the idea that endogenous pyrogen is derived primarily from leukocytes is based on experiments demonstrating that leukocytes themselves are pyrogenic(2), and that endogenous pyrogen is absent from the serum of animals made leukopenic with nitrogen mustard(3). These observations have been cornerstones in the hypothesis that endotoxin fever is mediated solely through the release of endogenous pyrogen. Several observations, however, suggest that endotoxins may produce fever in the absence of EP. First, leukopenic animals with no demonstrable EP may have high fever following administration of endotoxin(3). Second, animals made tolerant to the pyrogenic action of endotoxin continue to have some fever in the absence of demonstrable EP(4). Finally, tolerance to the pyrogenic effects of endotoxin is not paralleled by diminution in the leukopenia(5). The apparent dissociation between EP and fever may be in part related to the fact that the passive transfer technique measures only relatively large quantities of EP, while much less EP may be needed to activate the thermoregulatory centers in the donor. One facet of this problem which has received insufficient attention, however, is the reactivity of the leukopenic animal to endotoxin *per se,* irrespective of release of EP. For example, leukopenic animals are more sensitive to the lethal effects of endotoxin and, at times, may also have a hyperthermic response to the pyrogenic stimulus(6). The present studies reexamine the effects of different doses of endotoxin on fever, release of EP, and lethality in normal and leukopenic animals.

Materials and methods. Male and female albino rabbits weighing 2-3 kg were housed in individual cages in an air-conditioned room. Food and water were withheld on the day of experiment. Temperatures were measured with an indwelling rectal resistance coil and a Telethermometer (Yellow Springs Instrument Co., Yellow Springs, Ohio) every 30 minutes for 8 hours after injection of endotoxin and 3-4 hours after injection of endogenous pyrogen. White blood counts and differential counts were performed in the usual manner. The endotoxin was *S. enteritidis* (Difco Laboratories, Detroit, Mich.) suspended in pyrogen-free sterile physiologic saline in concentration of 2.0 μg/ml. Injections were made through the marginal ear vein. Nitrogen mustard (Mustargen Hydrochloride, Merck Sharp & Dohme, Philadelphia), freshly mixed in pyrogen-free saline in a concentration of 1.0 mg/ml just prior to

* Aided by a Research and Training Grant from National Inst. Health, U.S.P.H.S.