

ies revealed that the antigen(s) reactive with autoantibodies and those reactive with isoantibodies have distinct electrophoretic mobilities. d) Gel diffusion precipitation tests of auto- and isoantibody containing sera appeared to yield reactions of non-identity.

1. Beutner, E. H., Djanian, A. Y., Geckler, R. C., Witebsky, E., *Proc. Soc. Exp. Biol. and Med.*, 1961, v107, 486.

2. Colover, J., *Brain*, 1954, v77, 435.

3. Ouchterlony, O., *Acta Path. et Microb. Scand.*, 1953, v32, 231.

4. Grabar, P., Williams, C. A., Jr., *Biochem. Bio-*

phys. Acta, 1955, v17, 67.

5. Waterhouse, J. P., *Proc. Royal Soc. Med.*, 1963, v56, 911.

6. Witebsky, E., Rose, N. R., Terplan, K., Paine, J. R., Egan, R. W., *JAMA*, 1957, v164, 1439.

7. Block, K. J., Wohl, M. J., Ship, I. L., Oglesby, R. B., Bunin, J. J., *Arthritis and Rheumatism*, 1960, v3, 287.

8. Anderson, J. R., Gray, K. G., Beck, J. S., Kinnear, W. F., *Lancet*, 1961, v2, 456.

9. Rose, N. R., Metzger, R. S., Witebsky, E., *J. Immunol.*, 1960, v85, 575.

Received July 9, 1964. P.S.E.B.M., 1965, v118.

Parotid Saliva-Serum Ratios of Ca^{47} in Man Following Intravenous Administration of the Isotope.* (30001)

I. ZIPKIN AND L. V. AVIOLI†

U. S. Department of HEW, PHS, National Institutes of Health, National Institute of Dental Research, Laboratory of Biochemistry, Bethesda, Md. and Division of Endocrinology, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey

The present study was designed to investigate the salivary transport of calcium in the human by determining the specific activity ratios of Ca^{47} in the blood and parotid saliva following intravenous injection of the isotope. Only Greenberg *et al*(1) appear to have reported on the blood to saliva specific activity ratios of Ca^{47} in the human. However, no details were given.

Methods. Five normal males, 21-39 years of age and 5 normal females, 24-36 years old received 10-15 μC Ca^{47} intravenously before breakfast. At frequent intervals during the next 24 hours, as shown in Table I, blood was taken from the contralateral antecubital vein, and parotid saliva was collected according to previously described techniques(2-4). Calcium was determined by flame photometry according to MacIntyre(5) and expressed in grams per 2.0 ml. Ca^{47} was determined on 2 ml of serum and parotid saliva in a well scintillation counter. Discriminator settings

were selected to give optimal statistical accuracy with a counting error no greater than 1%. Energies below 400 kev were eliminated by a single channel gamma ray spectrometer to exclude the gamma emission of Sc^{47} . In each instance, aliquots of the administered Ca^{47} test dose were also counted using identical geometric conditions. Specific activity in saliva and serum is defined as the fraction of injected dose of Ca^{47} per gram of calcium. These are plotted for both males and females in Fig. 1.

Results. For both males and females, the salivary calcium varied from 2.5 mg% to 3.3 mg%, and blood calcium from 9.2 mg% to 10.0 mg%. The ratio of saliva to serum specific activities of Ca^{47} of the males approached unity one hour after intravenous administration of the isotope and was maintained for 24 hours.

The mean salivary-serum specific activity ratios in the females, in general, approached unity during the 24-hour study. A high salivary-serum specific activity ratio of 1.78 was obtained in one patient (Table I) 30 minutes following Ca^{47} infusion. The females appeared in fact to "concentrate" Ca^{47} in the

* Supported in part from USPHS Grant AM 06404-03 and AEC Grant AT(30-1)-3174.

† National Institute of Medical Sciences Career Research Development Awardee, National Institutes of Health (6-K3-G-22, 676-01-A1).

TABLE I. Specific Activity of Ca^{47} in Human Saliva (SA) and Serum (SE).

Males*													
Hr†	SA	SE	SA	SE	SA	SE	SA	SE	SA	SE	Mean		Mean
											SA	SE	SA/SE
1	.84	.80	.88	.90	.68	.60	.61	.59	.70	.76	.74	.73	1.02
2	.65	.62	.69	.76	.51	.48	.40	.36	.55	.56	.56	.56	1.00
4	.44	.46	.53	.55	.41	.38	.25	.27	.43	.46	.42	.42	1.00
6	—	—	—	—	.39	.35	—	—	.39	.39	.39	.37	1.06
8	.36	.34	.40	.42	.29	.31	.22	.20	.36	.36	.33	.33	1.00
12	—	—	.35	.35	.28	.28	.16	.17	.32	.33	.28	.28	1.00
16	.28	.29	.30	.32	—	—	.15	.15	.28	.31	.25	.27	.92
24	.25	.23	.28	.25	.22	.22	.13	.14	.25	.26	.23	.22	1.04
Females*													
Hr†	SA	SE	SA	SE	SA	SE	SA	SE	SA	SE	Mean		Mean
											SA	SE	SA/SE
1/2	—	—	—	—	1.87	1.05	—	—	—	—	1.87	1.05	1.78
3/4	—	—	—	—	—	—	—	—	1.06	.93	1.06	.93	1.15
1	.88	.73	.84	.79	.87	.71	.75	.69	—	—	.84	.73	1.16
1 3/4	—	—	—	—	—	—	—	—	.66	.53	.66	.53	1.25
2	.66	.55	.67	.55	.60	.52	.53	.55	—	—	.62	.54	1.16
4	.45	.44	.42	.46	.46	.43	.45	.45	.58	.42	.47	.44	1.07
6	—	—	—	—	—	—	.43	.41	—	—	.43	.41	1.05
8	.36	.36	.34	.34	.42	.38	.38	.34	.37	.34	.37	.35	1.05
12	—	—	—	—	.32	.29	—	—	—	—	.32	.29	1.10
16	.25	.33	.28	.32	—	—	—	—	—	—	.27	.33	.82
24	.19	.22	.26	.23	.25	.23	.30	.24	.19	.21	.24	.23	1.05

* Males: 21-39 yrs of age; females 24-36 yrs of age.

† Time after intravenous injection of Ca^{47} .

parotid saliva for the first 2 hours following administration of the isotope with equilibrium being established during the subsequent 22-hour period. Except for an anomalously low specific activity ratio of 0.82 16 hours after administration of Ca^{47} , all succeeding ratios were no more than 10% greater than unity.

Discussion. It appears that an equilibrium is established between salivary and serum calcium within one hour after administration of Ca^{47} in the male. The females showed some tendency to "concentrate" Ca^{47} in the parotid saliva, which was greatest $\frac{1}{2}$ hour after injection of the isotope ($\text{SA/SE} = 1.78$). This value is, however, based on only one individual. The low ratio of 0.82, based on 2 females 16 hours after injection of Ca^{47} , is unexplained. The greater ability of the female to "concentrate" Ca^{47} in the parotid saliva within 2 hours after administration of the isotope is reminiscent of the phenomenon of sexual dimorphism in the salivary glands of rodents, where morphological or biochemical differences or both have been observed in the submaxillary glands of the rat, mouse and hamster as reviewed by Sreebny *et al*

(6), Bergen *et al*(7), Houssay *et al*(8), Shackleford *et al*(9), and Kronman(10). Sex differences in the salivary content of some electrolytes have also been observed in humans(11,12) particularly in calcium loading studies(12).

It is likely that the apparent ability of the parotid gland of the human female to "concentrate" Ca^{47} is relative. That is, the higher specific activity ratios of saliva to serum in the female may represent a rapid uptake of Ca^{47} in the parotid gland tissue presenting an unstable equilibrium. In addition, it is possible that the pool of calcium in the parotid gland is not readily miscible again representing an unstable equilibrium. A corollary index of the parotid gland to "concentrate" Ca^{47} would have been an analysis of the gland itself for both Ca^{47} and stable calcium. In the present study, this was not possible.

The ability of the human female to "concentrate" other ions has also been reported. Taggart *et al*(13) recorded saliva-serum ratios of 1.05-1.39 with a mean of 1.19 in 10 pregnant women receiving D_2O orally at various periods during gestation. Using orally administered tritiated water, Coppen *et al*

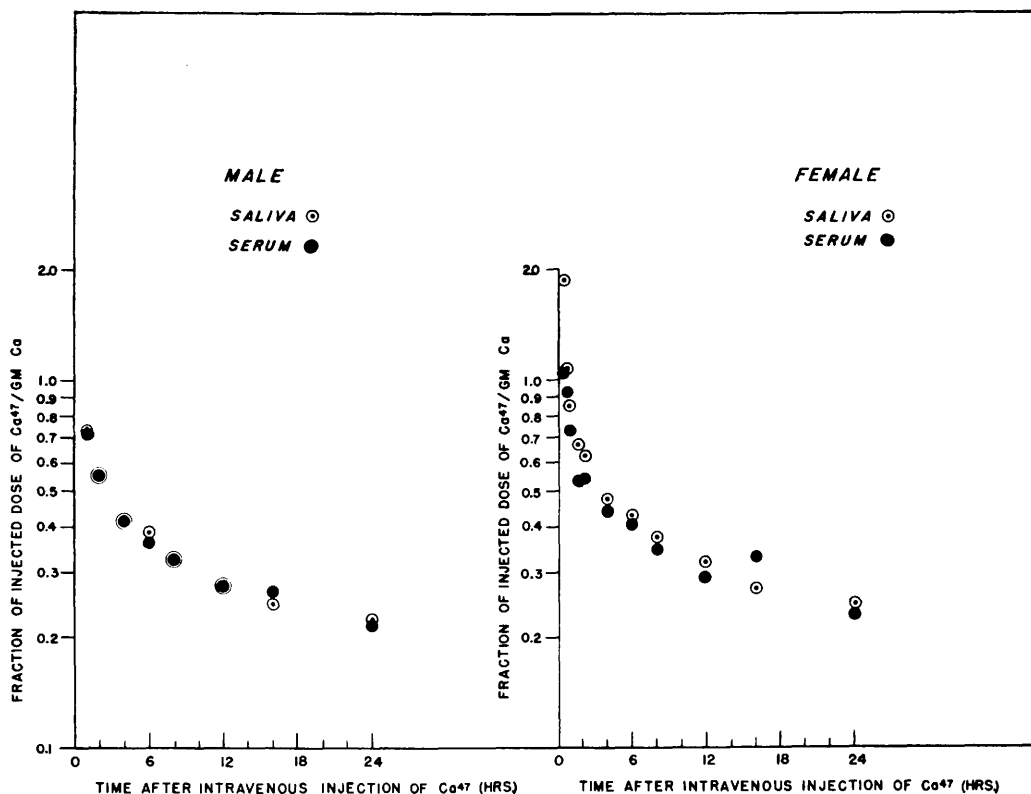


FIG. 1. Specific activity of Ca^{47} in human saliva and serum.

(14) found saliva-water, serum-water ratios of 0.99-1.01 with a mean of 1.00 in 3 normal males and 3 normal females. They interpreted their data to suggest that the salivary glands could distinguish between deuterium and hydrogen, but could not concentrate tritium.

Our present studies indicate that 2 hours after administration of Ca^{47} to normal adults, equilibrium is established between serum and saliva with regard to salivary transport. Thus, the monitoring of salivary radiocalcium, following an intravenous dose, may provide an index of serum radiocalcium, and would be particularly advantageous in those situations which preclude multiple venipunctures.

Summary. 1. Ca^{47} was administered intravenously to 10 normal adults, and serum and parotid salivas were obtained at intervals during a 24-hour period. 2. The parotid saliva-serum specific ratio

$$\text{S.A.} = \frac{\text{Fraction of administered } \text{Ca}^{47}}{\text{gm Ca}}$$

was near unity one hour after administration of the isotope to the 5 males. 3. The females appeared to "concentrate" Ca^{47} in the parotid saliva particularly during the first 2 hours after intravenous administration of Ca^{47} . All succeeding ratios were no more than 10% greater than unity.

1. Greenberg, E., Mertins, M., Weber, D., Corey, K., Kenney, P., Laughlin, J., *J. Nuclear Med.*, 1963, v4, 194.
2. Zipkin, I., Hawkins, G. R., Mazzarella, M. A., *Salivary Glands and Their Secretions*, L. M. Sreebny, J. Meyer, Eds., Pergamon Press, N. Y., 1964, p331.
3. Zipkin, I., Hawkins, G. R., *J. Dent. Res.*, 1964, v43, 1040.
4. Hawkins, G. R., Zipkin, I., *ibid.*, 1963, v42, 1015.
5. MacIntyre, I., *Biochem. J.*, 1957, v67, 164.
6. Sreebny, L. M., Meyer, J., Bachem, E., *J. Dent. Res.*, 1958, v37, 485.
7. Burgen, A. S. V., Emmelin, N. G., *Physiology of the Salivary Glands*, Williams & Wilkins Co., Baltimore, Md., 1961.
8. Houssay, A. B., Alberti, F. R., Cantis, M., Thierer, E., Rosner, J. M., *Acta Physiol. Latino*

Americano, 1961, v11, 190.

9. Shackleford, J. M., Klapper, C. E., *The Anat. Record*, 1962, v142, 495.

10. Kronman, J. H., *J. Dent. Res.*, 1963, v42, 825.

11. Zipkin, I., Wilk, A. L., Mantel, N., unpublished data.

13. Taggart, N., Hytten, F. E., *Nature*, 1959, v184, 457.

14. Coppen, A. J., Gibbons, J. L., *ibid.*, 1960, v186, 724.

Received July 14, 1964. P.S.E.B.M., 1965, v118.

Metabolism of Free Fatty Acids in Obese Humans.* (30002)

JOSIAH BROWN (Introduced by D. H. Solomon)

Department of Medicine, University of California School of Medicine, Los Angeles

Obesity in humans is of great clinical importance because of its frequency and association with diseases such as diabetes mellitus and arteriosclerotic heart disease. The frequency of familial association and the difficulty in achieving lasting weight reduction have led to suggestions that there is a metabolic defect in human obesity(1). No evidence is presently available to indicate that a metabolic defect is etiologic for obesity in humans but there is evidence that metabolic deviations are present in obese humans. Obese humans, compared to normals, tolerate fasting or a high fat diet with less ketonemia, less nitrogen loss and a slower fall in blood glucose(2). The insulin concentration in the blood of fasting obese subjects is higher than normal and the blood insulin response to a glucose load is exaggerated(3). Glucose uptake by the forearm tissue is elevated but is less responsive to insulin infusion than in normals(4). From studies of recovery of $C^{14}O_2$ in expired air, it has been concluded that oxidation of glucose to CO_2 may be depressed(5). The following studies were undertaken to provide further information on some aspects of FFA metabolism in obese humans. They indicate that in most obese subjects, there is a difference from normal in the disposal of a tracer dose of FFA following glucose administration.

Materials and methods. Studies were conducted on 8 normal volunteers and 5 obese but otherwise normal women (Table I). The

obese subjects were studied while in the hospital on a metabolic ward whereas only one of the normal subjects was studied while under hospital observation. No difference was observed between the studies on this subject and the other normal subjects.

Palmitic acid- C^{14} † was bound to human serum albumin‡ in a molar ratio of 2.5:1 by addition of KOH to the isotope, heating to $40^\circ C$ and addition of albumin which resulted in a clear solution. The final concentration of palmitate was approximately $10 \mu c$ per ml. Sterility was accomplished by filtration and established by culture. Analysis of the isotope by gas-liquid chromatography revealed only palmitic acid and caprylate; all the radioactivity was present in the palmitate.§

The palmitate oxidation study was performed by continuous measurement of all expired $C^{14}O_2$ for one hour after intravenous injection of $10 \mu c$ of 1- C^{14} palmitate(6). A helmet was placed over the head of the subject; a stream of air carried expired air through an ionization chamber and infrared CO_2 analyzer. The specific activity of expired $C^{14}O_2$ calculated for each 10-minute period was expressed as radioactivity (milli-volt-minutes) per millimole of CO_2 and plotted against time after injection of the isotope.

Blood samples were collected at intervals of one hour from an antecubital vein before and after the oxidation study. FFA were ti-

* Financial support for this investigation is derived from Grant 309 from The Nutrition Foundation and AM-1847 from U.S.P.H.S.

† Research Specialties Co., Berkeley, Calif.

‡ Cutler human serum albumin (salt poor) contains 0.02 M sodium caprylate.

§ Analysis kindly performed by Dr. James Mead.