one in serum. The extent of the interference is decreased by the use of petroleum ether during the extraction procedure and is less in 75% than in 65% acid/ethanol mixtures. The presence of 20α-hydroxypregn-4-en-3-one in peripheral circulation of female rats may contribute in part to the reported sex differences in concentration of serum corticosterone when measured fluorimetrically(8). Further, since 20α-hydroxypregn-4-en-3-one secretion is greatest during late proestrus(5), observed changes in corticosterone level during the estrous cycle(9,10) may be attributed to interference from this progestin.

A sensitive fluorimetric method can be adapted for measurement of small quantities of 20α-hydroxypregn-4-en-3-one present in ovarian tissue and venous blood. Differences between corticosterone and 20α-hydroxypregn-4-en-3-one in development time in 75% acid/ethanol suggests that these two steroids may be determined simultaneously in one sample by the use of calculations suggested by Stewart *et al*(11) for simultaneous measurement of corticosterone and cortisol.

*Summary.* A comparison of 20α-hydroxypregn-4-en-3-one and corticosterone reveals that their fluorescence properties are similar in concentrated sulphuric acid. Since rodent ovaries secrete 20α-hydroxypregn-4-en-3-one, this steroid may interfere with the assay of corticosterone by fluorimetric techniques; however, interference may be reduced by the use of a petroleum ether wash. It is suggested that a fluorescence method may be used as a sensitive assay for 20α-hydroxypregn-4-en-3-one.

1. Sweat, M. L., Anal. Chem., 1954, v26, 773.
2. Peterson, R. E., J. Biol. Chem., 1957, v225, 25.
3. Silber, R. H., Busch, R. D., Oslapas, R., Clin. Chem., 1958, v4, 278.
4. Eto, T., Masuda, H., Shzuki, Y., Hosi, T., Jap. J. Animal Reprod., 1962, v8, 34.
5. Wiest, W. G., Endocrinology, 1959, v65, 825.
6. Hilf, R., Burnett, F. F., Borman, A., Cancer Res., 1960, v20, 1389.
7. Loutfi, G., Peron, F., Dorfman, R. I., Endocrinology, 1962, v71, 983.
8. Kitay, J. I., ibid., 1963, v68, 818.
9. Callard, I. P., Callard, G. V., Anat. Rec., 1963, v145, 214.
10. Pincus, G., Hirai, M., Acta Endocrinol., 1964, v45, Suppl. 90, 191.
11. Stewart, C. P., Albert-Recht, F., Osman, L. M., Clin. Chim. Acta, 1961, v6, 696.

Received November 30, 1964. P.S.E.B.M., 1965, v118.

## Secretion of $Sr^{85}$ and $Ca^{47}$ in Human Saliva.* (29958)

JOSEPH SAMACHSON, VERNICE VANKINSCOTT AND HERTA SPENCER
*Metabolic Section, Veterans Administration Hospital, Hines, Ill.*

Several investigations have shown(1,2) that radiocalcium and radiostrontium have different rates of passage through various membranes and in different secretions of the body. Little is known about the relative secretion of the two radioisotopes in saliva. It has been reported recently(3) that a decrease of the body burden of $Sr^{85}$ in cats and mice can be attained more readily by using pilocarpine to stimulate the flow of saliva than by increasing the excretion of radiostrontium in urine. The increased secretion of $Sr^{85}$ in saliva is said to take place(3) with less loss of calcium than the excretion of $Sr^{85}$ in urine. The literature contains conflicting accounts concerning the effects of stimulating the secretion of saliva on concentration of calcium, some cited by Dreisbach(4). One group of investigators reported that concentration of calcium in saliva rose with an increase in flow rates(5). In his own work, Dreisbach(4) found that the concentration of calcium in saliva, obtained by pilocarpine stimulation in rats, decreased from an initial level of 6.4 mg% to less than a third of this value in one hour. He also found that rat salivary glands discriminated slightly in favor of Ca and against $Sr^{85}$ in secretion of

* Supported by research grant RH-00222 from Division of Radiological Health, U.S.P.H.S.

saliva(6). If $Sr^{85}$ secretion in man followed the same pattern as calcium and strontium secretion in rats, the use of stimulated secretion of saliva to increase the removal of radiostrontium did not seem to be promising. To obtain more information on the secretion of strontium and calcium in saliva in man, an investigation was conducted on the salivary secretion of $Sr^{85}$ and $Ca^{47}$. Preliminary results have been reported(7). More complete results are reported here.

*Material and methods.* Nineteen patients were maintained for study on the Metabolic Research Ward, where they received a constant low calcium diet that contained an average of about 200 mg Ca and 750 mg P per day. Ten additional patients received a general ward diet. Some of the patients on the Metabolic Ward received additional calcium in the form of calcium gluconate tablets. Six patients were studied with $Ca^{47}$ only, 6 with $Sr^{85}$ only, and 7 with both radioisotopes. Of the 13 patients who received $Ca^{47}$, 3 were studied on high calcium intake, 7 on low calcium intake, 2 on both diets, and 1 patient during starvation. Four patients received tracer doses intravenously, 8 orally, and 1 both orally and intravenously.

Of the patients studied with $Sr^{85}$, 7 were on low calcium intake and 6 on high calcium intake. Ten were studied after intravenous doses, 2 after oral doses, and 1 patient after both oral and intravenous doses.

Serum was analyzed for calcium and phosphorus once or twice weekly by the Clark-Collip modification of the Kramer-Tisdall method(8) and Fiske and SubbaRow method (9), respectively. The doses of intravenously injected $Sr^{85}$ and $Ca^{47}$ given as the chlorides ranged from 0.1 to 0.4 $\mu$c/kg. The $Sr^{85}$ was carrier free, while the $Ca^{47}$ contained a fraction of a milligram of stable calcium per dose. Doses were administered orally in slightly larger quantity, with 25 mg of stable Sr as carrier for $Sr^{85}$ and 25 mg of stable Ca as carrier for $Ca^{47}$. Intravenous doses were given to the patients before breakfast, while oral doses were administered with breakfast to ensure mixing of the isotope with food. Salivation was induced by the chewing of small blocks of wax. Chewing began 10 minutes before the scheduled time for blood sampling. Collection of saliva was begun 5 minutes later and continued until 5 minutes after the blood sample was obtained. Blood samples were obtained 1, 4, 8 and 24 hours after radioisotope administration, then daily for 5 days and every other day during the following 6-day period. Samples of saliva were obtained to correspond to plasma samples, the collection of saliva being discontinued when the radioisotope level became too low for accurate counting.

Ten patients on the general ward received $Ca^{47}$ intravenously and samples of saliva were collected by aspiration from the oral cavity. These studies were of several hours duration.

Determinations of $Sr^{85}$ were made on samples of saliva and plasma in a well-type $\gamma$-scintillation counter. $Ca^{47}$ was counted in a pulse height analyzer set for 1.3 Mev so as to exclude the counts of $Sc^{47}$ resulting from disintegration of $Ca^{47}$.

*Results.* Fig. 1 illustrates the changes in concentration of $Ca^{47}$ and $Sr^{85}$ in plasma and saliva after intravenous administration of both radioisotopes to the same patient. $Ca^{47}$ and $Sr^{85}$ plasma levels were practically identical during the first day. The level of $Ca^{47}$

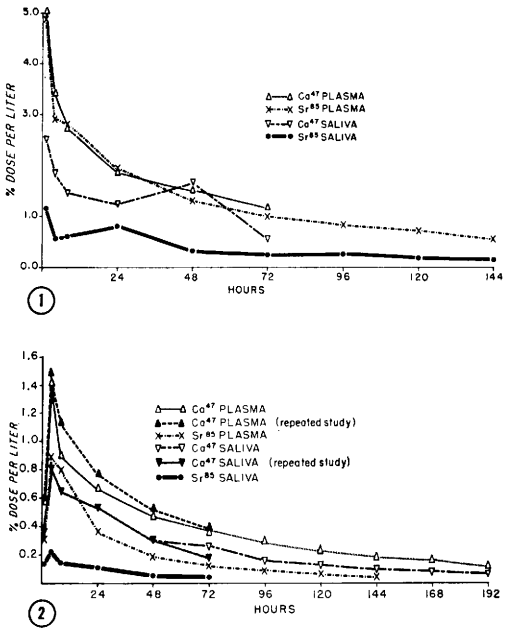


FIG. 1. Concentration of $Ca^{47}$ and $Sr^{85}$ in plasma and saliva after intravenous administration.

FIG. 2. Concentration of $Ca^{47}$ and $Sr^{85}$ in plasma and saliva after oral administration.

TABLE I. $Ca^{47}$ Concentration in Saliva and Plasma (Oral Administration of $Ca^{47}$).

| Patient B.C. | Hr after administration | $Ca^{47}$, % dose/l Saliva | Plasma | Saliva/Plasma |
|---|---|---|---|---|
| High Ca diet | 1 | .39 | .42 | .93 |
| | 4 | .69 | .84 | .82 |
| | 8 | .26* | .74 | — |
| | | 1.72* | | |
| | 24 | .57 | .60 | .95 |
| | | | Avg | .90 |
| Low Ca diet | 1 | .87 | .83 | 1.05 |
| | 4 | 1.26 | 1.53 | .82 |
| | 8 | 1.04 | 1.35 | .77 |
| | 24 | .80 | .85 | .94 |
| | 48 | .66 | .75 | .88 |
| | 72 | .57 | .66 | .86 |
| | | | Avg | .89 |

* Heavy sediment.

in saliva was approximately ⅔ the plasma level while the level of $Sr^{85}$ in saliva was only ⅓ the plasma level.

Fig. 2 illustrates in similar fashion the concentrations of $Sr^{85}$ and $Ca^{47}$ in plasma and saliva after oral administration to another patient. In this case the $Ca^{47}$ level of saliva was approximately half the level of $Ca^{47}$ in plasma while the $Sr^{85}$ level of saliva was again approximately ⅓ the $Sr^{85}$ level of plasma.

Table I lists results obtained for comparative determinations of $Ca^{47}$ in saliva and plasma in a patient who was studied on both a high and low calcium diet. There was some variability, but the average saliva/plasma ratio was 0.9, the same on both diets. Levels of both saliva and plasma were lower on the high calcium diet because a smaller percentage of $Ca^{47}$ was absorbed on this diet. However, the ratio of $Sr^{85}/Ca^{47}$ for saliva/plasma was the same for both diets. In general, there was no difference in the ratio between high and low calcium diets, just as there was no difference in the ratio between oral and intravenous doses once absorption had taken place. Results are therefore grouped together for high and low calcium diets as well as for oral and intravenous doses.

Occasional samples, like the 8-hour sample of patient B.C. on the high calcium diet, formed precipitates and gave erratic counts. The high counting material was found to be in the precipitate, the supernatant fluid retaining few counts. Analysis of one precipitate showed that it contained approximately twice as much Ca and P, the values being 1.44 mg Ca and 0.63 mg phosphorus. The supernatant fluid, however, contained 0.5 mg% Ca and 4.8 mg% P. Several samples of saliva which did not form a precipitate had 6 mg% Ca.

Table II shows plasma levels and saliva/plasma ratios in a patient who received 2 separate doses of $Sr^{85}$ intravenously and one intravenous dose of $Ca^{47}$. During the first day, $Sr^{85}$ and $Ca^{47}$ plasma levels were almost identical, although by 8 hours, the $Sr^{85}$ plasma level in both studies was definitely lower than the $Ca^{47}$ level. The rapid decrease of $Sr^{85}$ in the plasma of this patient reflected rapid excretion of $Sr^{85}$ in the urine. Both $Sr^{85}$ studies gave the same strikingly consistent saliva/plasma ratios. The $Ca^{47}$ study gave a saliva/plasma ratio twice as great. The difference between $Ca^{47}$ and $Sr^{85}$ ratios was highly significant, $P<0.001$.

Table III lists saliva/plasma ratios of the 2 radioisotopes in 7 patients. The ratio for $Ca^{47}$ was definitely higher than the ratio for $Sr^{85}$ and the average of the $Sr^{85}/Ca^{47}$ ratios was 0.45, the range being from 0.35 to 0.53, and the 95% confidence limits for the mean ratio of the 2 radioisotopes from 0.391 to 0.503.

Table IV summarizes results of saliva/plasma ratios of $Ca^{47}$ and $Sr^{85}$ in all patients. The average ratio for $Ca^{47}$ was 0.64 for the

TABLE II. Plasma Levels and Saliva/Plasma Ratios After Intravenous Doses of $Sr^{85}$ and $Ca^{47}$ (Patient K.B.).

| Time, hr | $Sr^{85}$ | $Ca^{47}$ | $Sr^{85}$ |
|---|---|---|---|
| | A. Plasma levels | | |
| 1 | 3.87 | 3.97 | 3.71 |
| 4 | 2.95 | 3.03 | 2.97 |
| 8 | 2.09 | 2.46 | 2.25 |
| 24 | 1.33 | 1.73 | 1.50 |
| 48 | .79 | 1.33 | .82 |
| 72 | .53 | .98 | .58 |
| | B. Saliva/plasma ratios | | |
| 1 | .21 | .40 | .18 |
| 4 | .19 | .35 | .18 |
| 8 | .22 | .36 | .20 |
| 24 | .20 | .43 | .21 |
| 48 | .18 | .42 | .23 |
| 72 | .19 | .44 | .24 |
| Avg | .20 | .40 | .21 |

TABLE III. Saliva/Plasma Ratios of $Sr^{85}$ and $Ca^{47}$ in Different Patients.

| Patient | $Sr^{85}$ | $Ca^{47}$ | $Sr^{85}/Ca^{47}$ |
|---|---|---|---|
| J.H. | .30 | .69 | .43 |
| R.I. | .20 | .57 | .35 |
| K.B. | .20 | .40 | .50 |
| T.K. | .30 | .57 | .53 |
| H.O. | .26 | .61 | .43 |
| L.S. | .22 | .54 | .41 |
| K.W. | .32 | .67 | .48 |
| Avg | .257 ±.019* | .579 ±.036* | .447 ±.023* |

* S.E. of mean.

13 patients in whom the flow of saliva was stimulated by chewing wax, the range being from 0.40 to 0.93. The average ratio for $Ca^{47}$ in the 10 patients in whom saliva samples were obtained by aspiration was 0.52, significantly less, at the 0.05 level, than the ratio for the patients who chewed wax. In these samples the lowest ratio was 0.40 and the highest was 0.60. The ratio for $Sr^{85}$ in 13 patients was 0.27, the range being from 0.20 to 0.32. The difference between the values for $Ca^{47}$ and $Sr^{85}$ was highly significant, $P << 0.001$.

*Discussion.* The results show unequivocally that, when secretion of saliva was stimulated by chewing wax, $Ca^{47}$ was secreted into the saliva at a higher concentration than $Sr^{85}$ relative to the plasma, the ratio favoring $Ca^{47}$ by more than 2 to 1. The saliva/plasma ratio for $Ca^{47}$ was more variable than that for $Sr^{85}$, the coefficients of variation being 22% and 15%, respectively. The value of 6 mg% for stable calcium concentration of saliva is in accord with the mean of $Ca^{47}$ values of saliva/plasma ratios of 0.58 (Table III), and 0.64 (Table IV), as all patients had plasma levels of calcium of about 10 mg%; this suggests that the average specific activity of $Ca^{47}$ in saliva and plasma was about the same. These values also agree with Dreisbach's value of 3.2 $\mu$Eq/g for stable calcium in rat saliva(4) and with his average of 1.41 mM/l for human saliva(10), and values of 1.3-1.7 mM/l for human saliva reported by Becks and Wainwright(11). The range of values for calcium in saliva reported in the literature corresponds to the variability of the $Ca^{47}$ ratios, which ranged from 0.40 to 0.93 (Table IV).

In some cases samples of saliva and plasma were obtained 15 to 30 minutes after administration of the dose. Some saliva values after either orally or intravenously administered doses were low at 15 minutes and some were low at 30 minutes, although many patients at 30 minutes and all patients at 1 hour showed no evidence of a further lag in secretion. Under conditions where saliva secretion was stimulated it appears that the lag may have been 15 to 30 minutes. It may have been greater in cases where saliva was collected by aspiration, as the amounts of saliva obtained in this manner were small and the calcium in this saliva may have come directly from calcium stored in the salivary glands(4).

The saliva contained on an average ¼ the concentration of $Sr^{85}$ in plasma. As the clearance of $Sr^{85}$ in urine is ordinarily more than 3 times the clearance of $Ca^{47}$(12), a liter of saliva may contain no more $Sr^{85}$ than 1/12 of a liter of urine. This fraction will vary with amount of urine excreted, with the calcium in the diet and in the urine, and with amount of $Sr^{85}$ secreted into saliva. In cases of prolonged stimulation of saliva flow by pilocarpine, it is likely that the concentration of $Sr^{85}$ in saliva may even decrease, as was found by Dreisbach to be the case with calcium in rats(4). It is clear that if 12 liters of saliva must be secreted to remove as much $Sr^{85}$ as is excreted in one liter of urine, it is highly impractical to use the secretion of saliva as a means of lowering the body burden of radiostrontium.

*Summary.* In a study of secretion of $Sr^{85}$

TABLE IV. Average Saliva/Plasma Ratios of $Ca^{47}$ and $Sr^{85}$.

| | $Ca^{47}$ (wax chewing) | $Ca^{47}$ (aspiration) | $Sr^{85}$ (wax chewing) |
|---|---|---|---|
| No. patients | 13 | 10 | 13 |
| Avg saliva/plasma | .638 | .515 | .267 |
| S.E. | .040 | .022 | .012 |

and of $Ca^{47}$ in saliva, the ratio of saliva concentration to plasma concentration varied from 1/5 to 1/3 for $Sr^{85}$ and from 2/5 to almost unity for $Ca^{47}$. The average ratios favored secretion of $Ca^{47}$ by a factor of more than 2, the difference in secretion of the 2 isotopes being highly significant. Values for $Ca^{47}$ were in accord with the levels of stable calcium found by us in saliva and previously reported by others. No difference in $Sr^{85}$/$Ca^{47}$ ratios of saliva in relation to plasma was found between patients on low and on high Ca intake. The high degree of discrimination of the salivary glands against the secretion of $Sr^{85}$ contrasts with the action of the kidneys in favoring secretion of $Sr^{85}$ over that of radiocalcium by a factor of approximately 3 to 1.

1. Samachson, J., Kabakow, B., Spencer, H., Laszlo, D., Proc. Soc. Exp. Biol. and Med., 1959, v102, 287.
2. Samachson, J., Kabakow, B., Spencer, H., ibid., 1960, v103, 570.
3. Setala, Kai, Naturwissenschaften, 1962, v13, 302.
4. Dreisbach, R. H., Am. J. Physiol., 1959, v196, 645.
5. Chauncey, H. H., Lisanti, V. F., Winer, R. A., Proc. Soc. Exp. Biol. and Med., 1958, v97, 539.
6. Dreisbach, R. H., ibid., 1959, v100, 719.
7. Samachson, J., Spencer, H., Fed. Proc., 1964, v23, 407.
8. Clark, E. P., Collip, J. B., J. Biol. Chem., 1925, v63, 461.
9. Fiske, C. H., SubbaRow, Y., ibid., 1925, v66, 375.
10. Dreisbach, R. H., J. Dent. Res., 1960, v39, 1133.
11. Becks, H., Wainwright, W. W., J. Dent. Res., 1946, v25, 267.
12. Samachson, J., Spencer-Laszlo, H., J. Appl. Physiol., 1962, v17, 525.

Received November 30, 1964. P.S.E.B.M., 1965, v118.

## A Plasma Factor Capable of Altering Triglyceride.* (29959)

BERNARD A. SACHS AND LILA WOLFMAN
*Endocrine Service, Medical Division, Montefiore Hospital and Medical Center, New York City*

A technique for thin-layer chromatography of blood lipids, recently reported from this laboratory, described 2 triglyceride fractions of different mobilities(1). Further studies of the possible origin of these fractions resulted in the finding of a hitherto unknown activity in human plasma capable of altering the Rf-value of added triglyceride on the thin-layer plates.

*Materials and methods.* Venous blood in the post-absorptive state was collected from 5 normal individuals and 12 patients with disorders of lipid metabolism into heparinized test tubes which were immediately placed in an ice-bath, centrifuged and sampled as rapidly as possible. One ml of each plasma sample was added to a 25 ml volumetric flask, approximately half-filled with Bloor's reagent (ethyl alcohol:ethyl ether, 3:1), following which one drop of triolein† (approximately 12 mg) was added from a capillary pipette. Plasma alone and triolein alone were similarly treated. The mixtures were shaken for 10 minutes at room temperature in a shaking machine, made up to mark and filtered. Ten ml of each filtrate was evaporated to dryness and taken up in 2 ml chloroform. Ten $\mu$l of each were sampled for thin-layer chromatography in a system which uses as solvents, first, n-propanol:$NH_4$ OH for 3 cm, and then, chloroform:benzene to 10 cm, as previously described(1).

In other experiments, the protocol was altered 1) by varying the plasma sample per 25 ml of Bloor's reagent from 0.01 ml to 1 ml, with the difference between the volume used and 1 ml made up with saline, 2) by varying the amount of Bloor's extract derived from the plasma from 0.5 ml to 15 ml with the volume adjusted to 15 ml with Bloor's

* Supported in part by grants from Nat. Inst. of Arthritis & Metab. Dis., U.S.P.H.S. and from G. D. Searle & Co.

† Mann Research Laboratories, CP > 90%.