STUDIES ON THE ORAL ADMINISTRATION OF SALIVA-
PAROTIN-A IV. ORAL AND INTRAIESTINAL
ADMINISTRATION OF ^{131}I -LABELLED SALIVA-
PAROTIN-A TO RATS

CHIAKI MORIWAKI AND HIROSHI MORIYA

*Laboratory of Physiological Chemistry, Faculty of Pharmaceutical
Sciences, Tokyo College of Science, Tokyo*

Yosoji ITO

*Department of Physiological Chemistry, Faculty of Pharmaceutical
Sciences, University of Tokyo, Tokyo*

SYNOPSIS

Radioactive iodine labelled saliva-parotin-A (^{131}I -SPA) was administered to rats by forced feeding or by injection into the ligated intestine in order to study its passage across the intestine. Higher radioactivity localization was found in the kidney as compared to control rats given KI- ^{131}I in the same method. The presence of ^{131}I -labelled macromolecules in serum was elucidated by the following procedures: phosphotungstic acid precipitation, radio-paper-chromatography and gel filtration with Sephadex G-75. From the results of the present investigation and those reported previously in this proceeding, the possibility of passage of saliva-parotin-A(SPA) across the intestine was raised.

There are various reports on the passage of proteins across the intestine as described previously (Ito *et al.*, 1965a), and many papers concerned with intestinal absorption of the enzyme proteins have also been published recently (Martin *et al.*, 1957 and 1962; Smyth *et al.*, 1961, 1962a, b and 1964; Miller *et al.*, 1960a and b; Avakian, 1961 and 1964; Kabacoff *et al.*, 1963). In these investigations the absorption of proteins administered in the digestive system was confirmed by immunochemical analysis and/or by detection of radioactivity, or the pigments used for labelling the sample protein in the body.

In the preceding papers it was reported that the physiological responses caused by the oral administration of a large amounts of SPA were analogous to those evoked by intravenous injection (Ito *et al.*, 1965a and b). Furthermore, the effects of human gastric and intestinal juices and liver on the biological activity of SPA were studied. Such activity of SPA was found to be unaffected by intestinal juice and rabbit liver (Ito *et al.*, 1966).

The present paper deals with further investigation of oral and intrainestinal administration of ^{131}I -SPA to assure the passage of SPA across the intestine.

MATERIALS AND METHODS

Preparation of ^{131}I -SPA

^{131}I -SPA was prepared as described previously (Ito *et al.*, 1966). ^{131}I -SPA of $1\mu\text{C}/\text{mg}$ was used in this experiment.

Oral administration of ^{131}I -SPA

Female Donryu strain rats, weighing 150g were fasted for 24 hrs., and forcedly fed 2.0 mg of ^{131}I -SPA (4.7×10^5 cpm) dissolved in 0.5 ml of distilled water, through a stomach tube. At 1, 2 and 3 hrs. after administration, 3 rats were sacrificed at each hour by exsanguination from the carotid artery. Four rats were injected intraperitoneally with 0.5 ml of the saline solution containing 0.001 M potassium iodide (KI) once a day and drank 0.001 M KI aqueous solution, *ad libitum*, during 3 days before the experiment. They were then administered 2 mg of ^{131}I -SPA orally and sacrificed 3 hrs. later. Total radio activity in the digestive tract, liver, kidney, spleen, thyroid and serum were counted with well-type scintillation counter ($2\phi'' \times 2$) and the percentage to the total radioactivity administered was obtained.

To 0.5 ml of serum, 0.2 ml of 0.1 M KI and 0.7 ml of 4%(w/v) phosphotungstic acid were added and the mixture was adjusted to pH 2 with n-hydrochloride. The protein precipitate was separated by centrifugation, washed 3 times with acidic phosphotungstic acid, and its radioactivity was counted.

Radio-paper-chromatography of the serum was performed as described in the preceding paper (Ito *et al.*, 1966) and the radioactivity distribution along the Rf value was estimated.

Intraintestinal administration of ^{131}I -SPA

Nine rats which received KI preliminary treatment were starved for 24 hrs. and injected with 2.6 to 3.0 mg of ^{131}I -SPA (1.3 to 2.0×10^6 cpm) dissolved in 0.2 ml saline solution, into the ligated loop of the jejunum according to Danforth and Moore (1959). As the control experiment, 0.001 M KI- ^{131}I (1.0 to 3.0×10^6 cpm) was given to 4 rats in the same method.

The animals were sacrificed by bleeding at 3 hrs. after the intraintestinal injection and then the count of radioactivity in tissues, the serum fractionation with phosphotungstic acid and radio-paper chromatography of the serum were performed as in the case of oral administration. Furthermore, the serum was submitted to gel filtration with Sephadex G-75 (1.3×2.5 cm column) using 0.1 M phosphate buffer (pH 7.0) as elution solvent and the radioactivity and optical density (at 280 m μ) in each fraction were measured.

EXPERIMENTAL RESULTS

As shown in Table 1, the absorption per cent of radioactivity, calculated from the radioactivity remaining in the digestive system or in the ligated intestine, was 60 to 75% in ^{131}I -SPA administered rats at 3 hrs. A much higher absorption rate was observed in the case of KI- ^{131}I administration. ^{131}I uptake in the thyroid was remarkably decreased by KI preliminary treatment, and it was confirmed that this treatment was effective in blocking thyroid function. There was a significant difference between radioactivity distribution in the kidney of the ^{131}I -SPA treated rat and that of the KI- ^{131}I administered rat ($p=0.05$).

The radioactivity in the whole serum was about 10% of the total administered radioactivity (Table 1). In the case of ^{131}I -SPA administration, 15 to 25% of the radioactivity in serum was insoluble in phosphotungstic acid (Table 2). Contrary to this, less than 8% of the radioactivity in serum was found in the protein precipitate in KI- ^{131}I administration. The insolubility of ^{131}I -SPA in the reagent

was ascertained from the experiment in which ^{131}I -SPA was mixed with normal rat serum and kept for 3 hrs. at room temperature; 91.2% of its radioactivity was found in the precipitate formed with phosphotungstic acid (Table 2).

Table 1. Radioactivity distribution in tissues after oral and intrainestinal administration of ^{131}I -SPA

Administration	Sample	No. of rats	Time min.	Absorption %* ²	RA in each tissue/Total administered RA (%)				
					Liver	Kidney	Spleen	Thyroid	Serum* ³
Oral	^{131}I -SPA	3	60	52.2±8.2	3.2±0.7	3.1±0.3	0.3±0.05	4.5±1.2	8.0±1.0
		3	120	53.9±9.0	3.0±1.0	0.9±0.3	0.3±0.1	5.4±1.0	11.4±1.5
		3	180	71.6±3.6	3.3±0.9	1.1±0.3	0.4±0.1	8.4±1.8	9.8±1.1
		4* ¹	180	61.6±6.6	2.9±1.2	1.1±0.1	0.1±0.05	0.4±0.1	10.4±0.7
Intrainestinal	^{131}I -SPA	9* ¹	180	75.2±6.8	2.1±1.1	1.3±0.2	0.2±0.1	0.2±0.1	10.6±1.3
	KI- ^{131}I	4* ¹	180	98.8±0.7	1.9±0.6	0.6±0.1	0.2±0.1	0.4±0.2	11.6±0.7

*¹ These rats were given KI preliminary treatment.

*² Absorption % = $\frac{\text{Total administered RA-RA in the digestive system}}{\text{Total administered RA}} \times 100$

*³ Total serum volume was estimated as 5 ml.

Table 2. Radioactivity in the acidic phosphotungstic acid precipitate of rats serum

Administration	Sample	No. of rats	Time min.	RA in ppt. %
Oral	^{131}I -SPA	3	60	25.4±2.1
		3	120	14.1±5.4
		3	180	19.9±2.3
		4*	180	18.3±2.5
Intrainestinal	^{131}I -SPA	9*	180	15.0±2.4
	KI- ^{131}I	4*	180	7.7±1.3
Control (^{131}I -SPA added in normal rat serum)				91.2

* These rats were given KI preliminary treatment.

In the quantitative radio-paper-chromatographic analysis, considerable radioactivity was observed at the starting line (R_f -0.05 to 0.05) in the serum of rats which received ^{131}I -SPA orally or intrainestinally (Fig. 1-d, -e). In the case of ^{131}I -SPA only or ^{131}I -SPA added rat serum, almost all radioactivity was found in the same area (Fig. 1-a, -b), while radioactive iodine appeared in the region of R_f 0.45 to 0.75 (Fig. 1-c). Therefore, it might be supposed that there was ^{131}I -SPA like radioactive substance in the serum of rats administered ^{131}I -SPA. There was no radioactivity at the starting line in the serum of rats receiving KI- ^{131}I and the highest radioactivity distribution was found in the area of R_f 0.45 to 0.65 (Fig. 1-f).

^{131}I -SPA and normal rat serum were mixed and submitted to gel filtration with Sephadex G-75. The whole protein, which was represented by optical density at 280 $m\mu$, eluted in the effluent at 9 to 16 ml; 95.7% of the radioactivity

Fig. 1. Quantitative radio-paper-chromatographic analysis of ^{131}I -labelled substances in rats serum.

- a) ^{131}I -SPA.
- b) ^{131}I -SPA added rat serum.
- c) KI- ^{131}I added rat serum.
- d) Serum of rat administered ^{131}I -SPA (oral).
- e) Serum of rat administered ^{131}I -SPA (intraintestinal).
- f) Serum of rat administered KI- ^{131}I (intraintestinal).

was also found in that effluent. Meanwhile, in the case of normal rat serum with KI- ^{131}I , no radioactivity elution was observed in the protein fraction. The whole radioactivity was recovered in the fraction of 30 to 40 ml (Fig. 2). The results of gel filtration in the serum of rats injected with ^{131}I -SPA or KI- ^{131}I in the ligated intestine are shown in Table 3. In ^{131}I -SPA administration, 3% of the radioactivity in the serum was obtained in the protein fraction (9 to 16 ml), but such ^{131}I combined protein was not found in the serum of the KI- ^{131}I treated rats.

Fig. 2. Elution pattern of protein and radioactivity in gel filtration of rat serum with added ^{131}I -SPA or $\text{KI-}^{131}\text{I}$. (Sephadex G-75, 1.3×25 cm column, $0.1M$ phosphate buffer, pH 7.0)

Table 3. Per cent of protein and radioactivity eluted in 1 ml of effluent with gel filtration in the serum of rats administered intraintestinally with ^{131}I -SPA or $\text{KI-}^{131}\text{I}$

Effluent (ml)	Serum of rats given ^{131}I -SPA		Serum of rats given $\text{KI-}^{131}\text{I}$	
	OD ₂₈₀	Radioactivity	OD ₂₈₀	Radioactivity
0—9	—	—	—	—
9—10	25.2	0.5	19.7	—
10—11	34.9	0.5	40.5	—
11—12	20.9	1.1	20.3	—
12—13	8.9	0.2	10.1	—
13—14	3.2	0.4	3.2	—
14—15	1.4	—	1.4	—
15—16	0.8	0.2	0.2	—
16—17	0.3	—	—	—
17—18	0.1	—	0.2	—
18—19	0.2	0.1	—	—
19—20	—	—	—	—
20—25	—	0.1	—	0.4
25—30	—	0.1	—	0.5
30—35	—	0.6	—	1.3
35—40	—	17.1	—	17.7
40—45	—	1.5	—	0.2

(Sephadex G-75, 1.3×25 cm column, $0.1M$ phosphate buffer, pH 7.0)

DISCUSSION

Recently, many reports concerning the passage of various enzymes across the intestine and the efficacy of oral administration of proteases as anti-inflammatory agents have been presented (Martin *et al.*, 1957 and 1962; Smyth *et al.*, 1961 1962a, b and 1964; Miller *et al.*, 1960a and b; Avakian 1961 and 1964; Kabakoff *et al.*, 1963; McPherson *et al.*, 1964; Tidwell *et al.*, 1964). In these investigations the enzyme protein was labelled with ^{131}I (Smyth *et al.*, 1964; Martin *et al.*, 1957; Miller *et al.*, 1960a and b; Avakian, 1961), dimethylaminonaphthalene sulfonyl chloride (Smyth *et al.*, 1961), or dinitrophenol (Smyth *et al.*, 1962b). Its absorption was studied by tracing the labelled substance in the body. Though the existence of the marker substance in serum does not mean the presence of the administered protein, it may be considered that the orally administered protein or its partially digested products are absorbed from the intestine; if it is certain that the marker substance in serum is combined with protein and can be proved that it does not combine easily with protein in the body. Furthermore, the observation of specific biological responses or the analytical confirmation of protein in the body after its administration into the digestive tract are also evidence supporting the intestinal absorption of protein.

In this experiment, SPA was labelled with ^{131}I and administered to rats by forced feeding or injection into the ligated intestine. Radioactivity was found in various tissues; high localization in the kidney and liver and low distribution in the spleen were observed. Ito *et al.* (1958) studied previously the localization of ^{131}I -SPA after intravenous injection and reported that the largest amount of radioactivity was found in the kidney while comparatively low distribution was found in the liver and spleen. The present data generally agree with these findings, except for distribution in the liver. This disagreement might be due to differences in the method of administration. There was little radioactivity in the kidney after the intrainstestinal administration of $\text{KI-}^{131}\text{I}$, therefore it may be presumed that the rat given ^{131}I -SPA absorbed not only radioactive iodine but some other substances tagged with ^{131}I .

About 10% of the total administered radioactivity was found in the serum after the administration of both samples, ^{131}I -SPA and $\text{KI-}^{131}\text{I}$. However, certain differences were detected in the radioactive substances in these sera. In the ^{131}I -SPA treated rats, 15 to 25% of the radioactivity in the serum was found in the phosphotungstic precipitate (Table 2) and 10 to 25% was observed at the starting line in radio-paper-chromatography (Fig. 1). In gel filtration with Sephadex G-75, 3% of the radioactivity was found in the serum protein fraction. It was considered that this elution was caused by neither the contamination of inorganic ^{131}I -labelled substance in the fraction nor the formation of labelled protein with radioactive iodine, since no radioactivity elution accompanied by serum protein was observed in the control experiment (Table 3). Meanwhile, in the serum of $\text{KI-}^{131}\text{I}$ treated rats there was less radioactivity in the protein precipitate and no radioactivity was found at the starting line in radio-paper-chromatography and the protein fraction obtained by the gel filtration.

These findings may indicate the existence of ^{131}I -labelled protein in the serum

of the rats receiving ^{131}I -SPA in the intestine. Haurowitz and Crampton (1952) and Yamamoto (1959) reported that no ^{131}I -bound protein was formed in the tissues, except the thyroid gland, after injection of inorganic radioactive iodine. The rats used in this investigation were preliminarily treated with KI and ^{131}I uptake by the thyroid was significantly decreased (Table I). Then it might be said that the thyroid function was considerably blocked in these rats. Furthermore, in considering that serum was obtained 3 hrs. after ^{131}I -SPA administration, it is assumed that there was no opportunity to form newly ^{131}I -combined protein in the body.

The results of this study can not confirm that the ^{131}I -labelled protein in serum was ^{131}I -SPA itself, however, the presence of such ^{131}I -labelled protein in the serum of control rats receiving KI- ^{131}I was hardly observed. Therefore it might be concluded that the radioactive substances passed across the intestine were not only radioactive iodine but some ^{131}I -bound macromolecules related to ^{131}I -SPA.

As mentioned in the previous papers, the physiological activity of SPA was stable in the digestive system (Ito *et al.*, 1966) and its responses to oral administration were analogous to the biological responses following subcutaneous or intravenous injection in rabbits (Ito *et al.*, 1965a and b).

After a through consideration of the results reported in the afore mentioned papers, it might be concluded that SPA is transmitted across the intestine while maintaining its physiological activity.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to Dr. S. Hirose, University of Chiba, for his helpful advice on the labelling technique. The authors are also indebted to Miss S. Akimoto, Tokyo College of Science, for her assistance in the experimental work.

REFERENCES

- Avakian, S. (1961). *New Eng. J. Med.* **264**, 764.
Avakian, S. (1964). *Clin. Pharm. Therap.* **5**, 712.
Danforth, E. and R. O. Moore (1959). *Endocrinology* **65**, 118.
Haurowitz, F. and C. F. Crampton (1952). *J. Immunol.* **68**, 73.
Ito, Y., Y.E. Kim and S. Tanaka (1958). *Endocrinol. Japon.* **5**, 277.
Ito, Y., H. Moriya and C. Moriwaki (1965a). *Ibid.* **12**, 298.
Ito, Y., C. Moriwaki and H. Moriya (1965b). *Ibid.* **12**, 305.
Ito, Y., C. Moriwaki and H. Moriya (1966). *Ibid.* **13**, 448.
Kabacoff, B.L., A. Wohlman, M. Umhey and S. Avakian (1963). *Nature* **199**, 815.
Martin, G.J., R. Brendel and J.M. Beiler (1957). *Am. J. Pharm.* **129**, 194.
Martin, G.J., J. Ehrenreich and N. Asbell (1962). *Exptl. Med. Surg.* **20**, 227.
McPherson, J.C., W.T. Moore, J.L. Pope and H.C. Tidwell (1964). *Proc. Soc. Exptl. Biol. Med.* **115**, 514.
Miller, J.M., D.R. Robinson and R.F. Williard (1960a). *Exptl. Med. Surg.* **18**, 348.
Miller, J.M., R.F. Williard and A.A. Polachek (1960b). *Ibid.* **18**, 352.
Smyth, R.D., R.M. Brennan and G.J. Martin (1961). *Am. J. Pharm.* **133**, 294.

- Smyth, R.D., R.M. Brennan and G.J. Martin (1962a). *Arch. int. Pharmacodyn.* **136**, 230.
- Smyth, R.D., A.R. Gennarh, R.M. Brennan and G.J. Martin (1962b). *Am. J. Pharm.*, cited by Smyth, R.D. *et al.* (1961).
- Smyth, R.D., R.M. Brennan and G.J. Martin (1964). *Ibid.* **22**, 46.
- Tidwell, H.C., J.C. McPherson, W.T. Moore, J.L. Pope and A. Freeman (1964). *Proc. Soc. Exptl. Biol. Med.* **116**, 720.
- Yamamoto, I. (1959). *Shonika Kiyo* **5**, 1054, 1063. (In Japanese)