STUDIES ON THE SALIVARY GLAND HORMONES
LABELED WITH I^{131}

3. EFFECT OF LIGATION OF SEVERAL BLOOD VESSELS ON
THE DEGRADATION AND DISTRIBUTION OF
 I^{131} -LABELED PAROTIN *IN VIVO**

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Previously it was reported that large localization of radioactivity was found in liver, kidney and spleen following the intravenous injection of I^{131} -labeled Parotin to rats (Tanaka and Ito, 1958). And subsequently it was also observed that the rate of degradation of I^{131} -labeled Parotin *in vivo* was remarkably decreased by the simultaneous injection of intact Parotin with the labeled Parotin to rats (Tanaka et al., 1958).

As simple hepatectomy causes a strong congestion in spleen, pancreas, and mesenteries, various modified methods have been reported including the functional evisceration by Russell (1942), the evisceration by Ingle *et al.* (1947) and physiological hepatectomy by Elgee and Williams (1954). It was reported that nephrectomy or physiological hepatectomy decreased the rate of degradation of I^{131} -labeled insulin in rats (Elgee *et al.*, 1953; Elgee and Williams, 1954).

The present report deals with the effect of ligation of several blood vessels on the degradation and distribution of I^{131} -labeled Parotin in rats.

MATERIALS AND METHODS

A Salivary Gland Hormone, Parotin (Ito, 1954) was labeled with radioactive iodine by the method similar to those previously reported (Tanaka *et al.*, 1958). The purity of I^{131} -labeled Parotin as expressed by the per cent of the total radioactivity found in trichloroacetic acid precipitable fraction was 98%.

Sixteen Wistar male rats (4 litter mates) were divided into 4 groups randomly in regard to the treatments as well as litters. In the control group, rats were only anesthetized with nembutal. In the three treated groups, (1) celiac artery, (2) bilateral renal vessels, (3) portal vein as well as celiac, superior mesenteric and inferior mesenteric arteries were ligated respectively. To be certain, blood vessel was ligated doubly with silk ligature. Approximately 20 mins. later, after the ligation of respective blood vessel or vessels, each rat was intravenously injected with 0.5 mg of I^{131} -labeled Parotin.

Received for publication June 23, 1958.

This work was supported in part by Grant in Aid for Scientific Research—Institutional Research from the Ministry of Education.

* Y. Ito: Studies on the Salivary Gland Hormones XXXII, XXXI: S. Aonuma and K. Yoshimura (1954). Japan. J. Pharm. Chem. **26**, 743.

Rats were sacrificed by exsanguination from the common carotid artery 30 mins. later, after the injection of the labeled Parotin. Approximately 10 mins. before exsanguination, rat was injected with 100 I.U. of heparin. One ml of plasma was collected by centrifugation and diluted with 9 ml of distilled water. About 100 mg of liver, kidney and spleen were weighed exactly, placed in planchet, added 1 ml of 2 N NaOH for digestion, dried under an infra-red lamp and the radioactivity was assayed with an end window Geiger Müller counter. Thyroid gland and 1 ml of the diluted plasma solution were similarly treated and the radioactivity was measured with the same counter. The remaining part of liver, kidney and spleen were weighed so as to measure the localization of the administered radioactivity in each organ. Separately, 1 ml of the diluted blood plasma solution was added with 1 ml of 10% trichloroacetic acid (TCA). After centrifugation, the precipitate was washed once with 2 ml of 5% TCA. Supernatants were pooled and neutralized with sodium hydroxide solution, and radioactivity in the precipitate and the pooled supernatant was assayed with a well-type scintillation counter.

RESULTS

Table 1 and 2 show the effect of ether and nembutal anesthesia on the rate of degradation of I^{131} -Parotin in rats as measured by the per cent of the total radioactivity in plasma found in TCA soluble fraction. As shown in Table 1,

Table 1. Effect of ether anesthesia on the degradation of I^{131} -labeled Parotin in rats*

Treatments	B.W. of rats (g)	Per cent of the total I^{131} in plasma found in TCA soluble fraction
Control	136	66
	133	62
	140	54
	156	62
		mean 61 **
Ether anesthesia	136	34
	117	26
		mean 30 **

* Rats were sacrificed 30 mins. later, after intravenous injection of 0.5mg of I^{131} -Parotin

** Significant difference ($F=47.5$, $P<0.01$)

Table 2. Effect of nembutal anesthesia on the degradation of I^{131} -labeled Parotin in rats*

Treatments	B.W. of rats (g)	Per cent of the total I^{131} in plasma found in TCA soluble fraction
Control	126	60
	122	69
	108	51
	87	59
		mean 59 **
Nembutal anesthesia	112	65
	123	69
	110	57
	104	56
		mean 62 **

* Rats were sacrificed 30 mins. later, after intravenous injection of 0.5mg of I^{131} -Parotin

** No significant difference ($F=0.38$, $P>0.1$)

the ether anesthesia remarkably decreased the rate of degradation of I^{131} -Parotin in rats. By nembutal anesthesia, however, the degradation of the labeled Parotin was not influenced at all (Table 2). Therefore the effect of the ligation of several blood vessels on the degradation and distribution of I^{131} -Parotin in rat was studied hereafter under nembutal anesthesia only.

Table 3 shows the effect of ligation of several blood vessels on the localization of radioactivity 30 mins. later, after the intravenous injection of I^{131} -Parotin to rats, and the statistical analysis of the results is summarized in Table 4. As a

Table 3. Effect of ligation of several blood vessels on the distribution of radioactivity after the injection of I^{131} -labeled Parotin to rats*

Per cent of the total administered I^{131} localized to each organ						
	B.W. (g)	Plasma	Liver	Spleen	Kidney	Thyroid
(A) Control	111	6.9	42	2.1	3.1	0.7
	121	6.7	46	1.7	2.7	0.9
	115	6.8	46	1.9	3.1	0.5
	103	6.2	46	1.7	4.3	0.8
	mean 112	6.6	45	1.9	3.3	0.7
(B) Ligation of celiac artery	119	7.1	71	0.1	4.4	0.14
	104	4.9	92	0.2	4.8	0.06
	110	6.7	69	0.1	3.9	0.19
	100	5.3	77	0.1	3.4	0.11
	mean 108	6.0	77	0.1	4.1	0.13
(C) Ligation of renal vessels	114	7.9	81	1.3	0.3	0.06
	105	8.1	75	0.9	0.5	0.01
	113	8.4	66	1.6	0.2	0.02
	101	7.5	80	1.0	0.1	0.05
	mean 108	8.0	76	1.2	0.3	0.04
(D) Ligation of celiac artery sup. mesenteric artery inf. mesenteric artery portal vein	117	65	10	0.6	7.6	0.09
	99	54	12	4.4	5.3	0.11
	105	72	4.6	0.2	7.6	0.05
	104	60	1.5	—	7.8	0.07
	mean 106	60	7.0	0.4	7.1	0.08

* Rats were sacrificed 30 mins. later, after the intravenous injection of 0.5mg of I^{131} -Parotin

Table 4. Result of the statistical analysis of Table 3

Organ	Groups compared	Fo	Level of significance
Plasma	A—B	2.6	>0.1
	A—C	32.6	<0.01
	A—D	218.6	<0.01
Liver	A—B	36.4	<0.01
	A—C	73.5	<0.01
	A—D	212.3	<0.01
Spleen	A—B	211.6	<0.01
	A—C	15.5	<0.01
	A—D	77.1	<0.01
Kidney	A—B	3.2	>0.1
	A—C	70.1	<0.01
	A—D	30.0	<0.01
Thyroid	A—B	41.1	<0.01
	A—C	57.5	<0.01
	A—D	50.1	<0.01

F0 calculated by F-test, c.f. $F_0^1 (0.01) = 13.7$ $F_0^1 (0.05) = 5.99$

matter of fact, very small amount of radioactivity was found in spleen after the ligation of celiac artery, in kidney after the ligation of renal vessels, and in liver and spleen after the ligation of portal vein as well as celiac, superior mesenteric and inferior mesenteric arteries. However, it is not clear whether it was due to the leakage of radioactivity through ligation or due to some other reasons that very small but not negligible radioactivity was found in those organs, especially in liver, even after the ligation of respective blood vessels.

Localization or retention of radioactivity in liver was remarkably increased following the ligation of celiac artery as compared with that of control group. Considering this large retention of radioactivity in liver, rather smaller radioactivity in plasma was understandably observed. And in spite of this smaller concentration of radioactivity in plasma, somewhat larger localization of radioactivity was found in kidney. Remarkably large localization or retention of radioactivity in liver was also found after the ligation of renal blood vessels and, in addition, fairly smaller localization of radioactivity was observed in spleen, and considerably larger concentration of radioactivity was found in plasma, as compared with those of control group. As if to reflect the very small localization of radioactivity in liver and spleen, extremely large amount of radioactivity was observed in plasma following the ligation of portal vein as well as celiac, superior mesenteric and inferior mesenteric arteries. And markedly large localization of radioactivity was observed in kidney.

Table 5. Effect of ligation of several blood vessels on the degradation of ^{131}I -labeled Parotin in rats*

Treatment's	Litter No.	B.W. (g)	Localization of ^{131}I in plasma**	^{131}I in TCA Supern.***	Degree of degradation****
(A) Control	1	111	6.9	73	5.0
	2	121	6.7	74	5.0
	3	115	6.7	73	5.0
	4	103	6.2	72	4.5
				mean	4.9
(B) Ligation of celiac artery	1	119	7.1	27	1.9
	2	104	4.9	19	0.9
	3	110	6.7	61	4.1
	4	100	5.3	51	2.7
				mean	2.4
(C) Ligation of renal vessels	1	114	7.9	24	1.6
	2	105	8.1	27	2.2
	3	113	8.4	55	4.6
	4	101	7.5	31	2.3
				mean	2.8
(D) Ligation of celiac artery, sup. mesenteric artery, inf. mesenteric artery, portal vein	1	117	65	1.1	0.7
	2	98	54	1.5	0.8
	3	105	54	1.2	0.9
	4	104	60	1.2	0.7
				mean	0.8

Significant difference A—B: $F=13.1$ ($P<0.025$), A—C: $F=10.9$ ($P<0.025$), A—D: $F=933$ ($P<0.01$)

* Rats were sacrificed 30 mins. later, after the intravenous injection of 0.5 mg of ^{131}I -Parotin.

** Per cent of the total administered radioactivity localized in plasma.

*** Per cent of the total radioactivity in plasma found in TCA soluble fraction.

**** Per cent of the total administered radioactivity localized in TCA soluble fraction of plasma.

Quite smaller localization of radioactivity was found in thyroid after the three kinds of ligation. And the smallest radioactivity was observed in the group where renal vessels were ligated.

Table 5 shows the influence of ligation of several blood vessels upon the degradation of I^{131} -labeled Parotin *in vivo* as estimated by measuring the radioactivity in TCA soluble fraction of plasma. If it was only aimed to seek the state of I^{131} in plasma, the mere measurement of the per cent of the total radioactivity in plasma found in non-protein fraction would be sufficient. Considering the different concentration of radioactivity in plasma according to each different kind of treatments, however, it seemed quite suitable to measure the per cent of the total administered radioactivity localized in the TCA soluble fraction of plasma, for the purpose of estimating the rate of degradation of I^{131} -Parotin *in vivo*. It was found that the rate of degradation of I^{131} -Parotin *in vivo* was greatly reduced after the ligation of celiac artery and also after the ligation of renal vessels. But the smallest degradation of I^{131} -Parotin was observed in the group where portal vein as well as celiac, superior mesenteric, and inferior mesenteric arteries were simultaneously ligated.

DISCUSSION

Hepatectomy, nephrectomy and viscerectomy as well as ligation of abdominal blood vessels of experimental animals require anesthesia. And for this purpose, it is desirable to choose the anesthetic which has not any significant effect upon the biological responses to be measured. Ether anesthesia has been often employed by many workers including Elgee and Williams (1954) who examined the effect of nephrectomy or physiological hepatectomy on the rate of degradation of I^{131} -labeled insulin in rats. So far as the degradation of I^{131} -labeled Parotin is concerned, ether anesthesia showed strong inhibitory effect, but nembutal did not.

The localization of radioactivity in liver was greatly increased following the ligation of celiac artery. This might be the result of the decreased rate of releasing the radioactivity by liver as compared with that of control animals. And in

Table 6. Effect of ligation of celiac artery on the distribution of radioactivity to liver 5 mins. later after the injection of 0.5 mg of I^{131} -labeled parotin to rats

Treatments	Rat No.	B.W. (g)	Per cent of the total administered I^{131} localized to liver
Control	1	123	78
	2	130	74
	3	124	66
	4	133	68
			mean 70*
Ligation of celiac artery	1	128	62
	2	115	63
	3	121	69
	4	107	58
			mean 63*

* No significant difference $F=5.4$ $P>0.05$

this connection, the effect of ligation of celiac artery on the localization of radioactivity 5 and 60 mins. later, after the intravenous injection of I^{131} -Parotin to rats was studied (Table 6 and 7). As shown in Table 6, little difference was

Table 7. Effect of ligation of celiac artery on the distribution of radioactivity to liver 60 mins. later, after the injection of 0.5 mg of I^{131} -labeled parotin to rats

Treatments	Rat No.	B.W. (g)	Per cent of the total administered I^{131} localized to liver
Control	1	112	14
	2	110	12
			mean 13*
Ligation of celiac artery	1	107	42
	2	103	37
			mean 39*

* Significant difference $F=90.1$ $P<0.01$

observed between the two groups (with and without the ligation of celiac artery), where rats were sacrificed 5 mins. later, after the injection of I^{131} -Parotin. On the other hand, the ligation of celiac artery produced remarkably larger retention of radioactivity in liver at the 60th mins. after the injection of the labeled Parotin as compared with that of non-ligated control group (Table 7). From these results, it was proved that following the ligation of celiac artery not only the labeled Parotin could enter the liver through portal vein system as rapidly as in the case of control animals but also the liver had still retained as much activity of accumulating I^{131} -Parotin as that of control rats. And it was also proved that the rate of disappearance of radioactivity from liver was greatly decreased along with the marked reduction of the rate of degradation of I^{131} -Parotin *in vivo* after the ligation of celiac artery. And from this it was strongly suggested that the rate of degradation of I^{131} -Parotin by liver was remarkably decreased by the ligation of celiac artery and therefore the rate of release of radioactivity by liver was greatly reduced.

Following the ligation of renal vessels, the localization of radioactivity in liver was markedly increased as compared with that of control group, although smaller amount of radioactivity was observed in spleen in comparison with that of control animals. From these it could be deduced that as one of the reduced or disordered functions through nephrectomy, the activity of liver to degrade I^{131} -Parotin was greatly decreased and therefore the remarkably delayed release of radioactivity by liver was observed after the ligation of renal vessels. Consequently, the decreased rate of degradation of I^{131} -labeled Parotin *in vivo* following the ligation of renal vessels was regarded as the results of both the absence of degradation of I^{131} -Parotin by kidney and the lowering of that by liver.

A close correlation between liver and kidney was also suggested in the case of ligation of celiac artery, because the increased localization of radioactivity in kidney was observed after the ligation of celiac artery as compared with that of control group, in spite of the decreased concentration of radioactivity in

plasma. Therefore, it was suggested that, in the case of ligation of celiac artery, the rate of degradation of the labeled Parotin *in vivo* was greatly lowered through the reduction of the rate of degradation of I^{131} -Parotin in liver and kidney as well as through the absence of the degradation of that in spleen. Similarly as the causes for the decreased rate of degradation of I^{131} -Parotin *in vivo* following the ligation of portal vein as well as celiac, superior mesenteric, and inferior mesenteric arteries, the reduced rate of degradation of the labeled Parotin by kidney must be considered in addition to the absence of the degradation of that by liver and spleen.

Concerning to the localization of I^{131} in the thyroid gland which is known to accumulate the iodide ion from plasma, the smallest localization of radioactivity in thyroid was observed after the ligation of renal vessels, although all of the three kinds of ligation greatly reduced the rate of localization of I^{131} in thyroid following the intravenous injection of I^{131} -labeled Parotin to rats as compared with that of control group. This may suggest that kidney is the most contributive to the deiodination of I^{131} -labeled Parotin *in vivo*.

SUMMARY

Effect of ligation of several blood vessels on the degradation and distribution of I^{131} -labeled Parotin in rat was studied. The degradation of the labeled Parotin *in vivo* as measured by the per cent of the total administered radioactivity found in non-protein fraction of plasma was remarkably decreased following the ligation of celiac artery or of renal vessels. However, the least degradation of I^{131} -Parotin *in vivo* was observed in the group where portal vein as well as celiac, superior mesenteric and inferior mesenteric arteries were ligated.

Significantly large retention of radioactivity was found in liver after the ligation of celiac artery and also after the ligation of renal vessels. It was suggested that very close correlations on the degradation and distribution of I^{131} -Parotin exist between liver and kidney which were recognized to be the main sites of degradation of the labeled Parotin *in vivo*.

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