from zero order reaction kinetics) for at least a fortnight at room temperature.

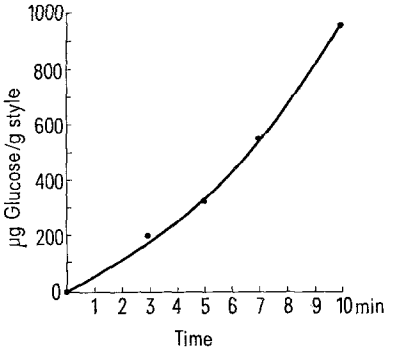
Laminaran is an algal carbohydrate polymer energy reserve which has as the major repeating structural unit the disaccharide laminarabiose (3-*O*-β-D-glucopyranosyl D-glucose). Glucans with (1 → 3), β-D-glucoidic linkages are found in some higher plants, e.g., barley and oats[4].

The major style carbohydrase activity, that of a (1 → 3)-β-D-gluconase, seems to be similar to laminaranases of bacterial origin[4]. In addition to the compounds shown in the Table, the material is inactive on sucrose, lactose, and guar gum. The amylase activity is chloride dependent, and the degree of polymerization required for action on (1 → 3)-β-D-glucans must be greater than two.

Action of the preparation on laminaran has a broad (4.5–5.5) pH optimum and temperature optimum at 37 °C. The laminaranase activity is not enhanced by chloride, iodide, fluoride, bromide and magnesium salts, nor by oxalate, nor calcium. It is precipitated by ammonium sulfate at concentrations greater than 25% saturation.

Carbohydrase activities of the crystalline style of the surf clam

Substrate	Glucosidic linkage	Relative turn-over number
Laminaran	β1,3	100
Cellulose	β1,4	3
Starch	α1,4; α1,6	2
Starch + 0.02 *M* NaCl	α1,4; α1,6	72
Gentiobiose	β1,6	1
Cellobiose	β1,4	7
Maltose	α1,4	0
Maltose + 0.02 *M* NaCl	α1,4	6
Laminarabiose	β1,3	1
Laminaratriose	β1,3	47
Laminaratetraose	β1,3	68
Laminarahexaose	β1,3	57
Nigeran	α1,3; α1,4	0.3
Pullulan	α1,4; α1,6	0



Generation of glucose from laminaran by the crystalline style of the surf clam.

We were able to 'mobilize' the enzyme by inserting a small metal rod into a portion of the style and spinning it slowly on a magnetic stirrer in a 0.06% solution of laminaran. An example of the results obtained is shown in the Figure. After about 10 min, however, the style itself begins to dissolve. The enzyme has also been successfully immobilized on glass. In a manner similar to the way the style of bivalves is able to lyse algae and bacteria, our preparations are able to lyse yeast cells. The cell population is reduced by 50% and then activity ceases.

In view of the demonstrated inhibitory effect of clam extracts on the growth of the sarcoma 180 tumor in Swiss albino mice, the inhibition of Krebs 2 carcinoma, and the carcinolytic activity on the human HeLa cell line by SCHMEER[3,5], we now take the opportunity to point out the similarity between this suspected anticarcinomic principle, or 'mercenene' of clams and our 'laminaranase' preparations. Both preparations are glycoproteins and are inactivated by boiling and therefore both may be enzymic in nature. Both are precipitated from aqueous solutions saturated with greater than 25% ammonium sulfate, and can be precipitated from solution by acetone. Activity apparently varies with the season, or conditions corresponding to the formation or dissolution of the style by the clam.

Zusammenfassung. Isolierung und Charakterisierung eines Enzyms aus *Spissula solidissima*, welches die Fähigkeit besitzt, gewisse Polysaccharide spezifisch zu spalten.

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[7] We thank W. E. GUILD for laboratory assistance, the Shelter Island Oyster Company, Long Island, N.Y. for samples, and the Sea Grant Advisory Service for their help. This study was supported in part by a Sea Grant from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

Extraction and Production of Renin and Angiotensin II Across Several Vascular Beds in Dogs[1]

In the present study we investigated the role of the lungs, the splanchnic region with exclusion of the spleen, the spleen and the liver in the inactivation or production of renin and angiotensin II by measuring plasma renin concentration (PRC) and plasma angiotensin II (PA II) in their efferent and afferent vessels. The study was performed on 4 intact dogs and on 7 dogs after exclusion of the liver, which is known to be a major site of renin[2–5]- and angiotensin II[6–8]-inactivation. In this study the words extraction, inactivation and production do not refer to specific mechanisms.

Materials and methods. 11 mongrel dogs, with an average weight of 24.9 ± 4.8 (S.D.) kg were anesthetized with i.v. sodium pentobarbitone 30 mg/kg body weight.

Table I. The mean percentage ratios of PRC across several vascular beds

Groups of dogs	Dog No.	Percentage ratio across			
		Lungs	Splanchnic region	Spleen	Liver
1	1–4	—	98.0 ± 12.0[a] (4)	92.3 ± 22.5[a] (4)	57.0 ± 23.3[b] (4)
2	5–11	97.8 ± 4.4[a] (6)	98.8 ± 2.1[a] (6)	101.8 ± 10.8[a] (5)	—

Catheters were introduced into the aorta (through the femoral artery), into the right ventricle of the heart (through the right jugular vein), into the common mesenteric vein, the splenic vein and into one of the hepatic veins (through a median laparotomy incision). In the first series of 4 dogs (dogs 1–4) all organs were left intact. Blood was drawn simultaneously from each site (except from the right ventricle in which no catheter was placed in these dogs) for the determination of PRC and PA II, 30 min after the introduction of the catheters. In the second group of 7 dogs, after the construction of a porto-caval shunt to avoid total obstruction of the portal circulation, the liver was excluded either by ligation of the hepatic arteries and portal vein (dogs 5–9) or by removal of the liver (dogs 10 and 11). Blood was drawn simultaneously from each site for determination of PRC and PA II, 30 min after the ligation of the hepatic blood vessels in dogs 5–9, and 30 min after the completion of the hepatectomy in dogs 10 and 11. As the values of both subgroups did not differ from each other, the results were computed together. Blood was collected in ice-cooled tubes and immediately centrifuged. The plasma was frozen at −20 °C until determination of PRC using the method of SKINNER[9] and determination of PA II using the method of VALLOTTON[10]. Renin metabolism across the vascular beds was studied by calculating the percentage ratios(%) of the PRC in their efferent vessel over the PRC in their afferent vessel:

$$\frac{\text{PRC in the efferent vessel}}{\text{PRC in the afferent vessel}} \times 100.$$

The inactivation or production of angiotensin II was studied in the same way.

Results and discussion. Table I shows the mean percentage ratios of PRC ± S.D. across the studied vascular beds for both groups of dogs. The values are close to and not significantly different from 100% ($P > 0.1$) for the pulmonary circulation, the splanchnic region with exclusion of the spleen and for the spleen. Others were unable to show inactivation of renin by the lungs[11] or by the total splanchnic territory[2–3] in dogs. The reported release or production of renin across the splanchnic territory[5,12] cannot be confirmed. The percentage ratio of PRC across the liver averaged 57%, indicating a percentage extraction of 43%. Percentage extractions ranging from 18.7% to 36.7% have been reported[2–5]. Table II shows the mean percentage ratios of PA II ± S.D. The PA II level in the aorta averaged 228% of the value in the right ventricle of the heart ($P < 0.05$), confirming important formation of angiotensin II by the lungs[13–15]. As reported by others in the dog by biological methods[6–8], the liver was found to be a site of angiotensin II-inactivation. The percentage ratio of 55.1% was obtained using the arterial PA II as the afferent PA II, as the portal venous PA II was not measured, nor were the arterial hepatic and the portal venous blood flow. However, the lower PA II in the common mesenteric vein and in the splenic vein indicate that the portal venous PA II was lower than the

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Table II. The mean percentage ratios of PA II across several vascular beds

Groups of dogs	Dog No.	Percentage ratio across			
		Lungs	Splanchnic region	Spleen	Liver
1	1–4	—	74.4 ± 11.2[b] (4)	75.5 ± 7.5[c] (4)	55.1 ± 26.9[b] (4)
2	5–11	228.0 ± 85.0[b] (6)	45.1 ± 18.3[d] (7)	43.4 ± 11.2[d] (5)	—

Values are means ± S.D. n, number of observations. P-value indicates difference from 100%, obtained by Student's t-test. [a] $P > 0.1$. [b] $P < 0.05$. [c] $P < 0.01$. [d] $P < 0.001$.

PA II in the hepatic artery, so that the percentage ratio of PA II across the liver was higher than 55.1%. Indeed, we demonstrated a significant extraction of angiotensin II across the spleen ($P < 0.01$) and across the splanchnic region with exclusion of the spleen ($P < 0.05$; group 1), for which no values are reported. After the exclusion of the hepatic circulation (group 2) the percentage ratio of angiotensin II decreased from 75.5% to 43.4% ($P < 0.01$) across the spleen and from 74.4% to 45.1% ($P < 0.05$) across the splanchnic region, indicating a significant increase in angiotensin II extraction. The data of this study do not allow to conclude if changes in haemodynamics account for this increase in the inactivation of angiotensin II within the spleen and the splanchnic region or if the absence of the liver is a stimulus to both vascular beds to increase the inactivation of angiotensin II.

Résumé. Cette étude démontre que la rénine est inactivée par le foie, et que les poumons, la rate et la région splanchnique ne la metabolisent pas. Les poumons forment de l'angiotensine II, tandis que le foie, la rate et la région splanchnique sont capables de l'inactiver. Après exclusion de la circulation hépatique, l'inactivation de l'angiotensine II par la rate et par la région splanchnique est augmentée.

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Melanogenesis from Tryptophan. Biogenetic Experiments with Harding-Passey Mouse Melanoma

The possibility that tryptophan is involved in melanogenesis has already been proposed. CHEN and CHAVIN[1] observed that, not only tyrosine, but tryptophan and other aromatic amino acids can be utilized as substrates from goldfish skin tyrosinase. NICOLAUS[2] reported that radioactive melanin was obtained in cuttlefish after injection of D,L-tyrosine-2-^{14}C or L-phenylalanine-^{14}C (U) or D,L-tryptophan [benzene ring-^{14}C (U)]. With the latter compound, the radioactivity of the ink sac gland was about 1/40 of that obtained when L-tyrosine-^{14}C (U) was injected. VISCONTINI and MATTERN[3,4], studying hydroxylation of tryptophan-3-^{14}C by means of tetrahydropterin in the presence of ferrous ions, observed that radioactive melanin was formed via 5-hydroxytryptophan.

Our previous research[5–7] on the action of polyphenol oxidase from potatoes, and from the *Psalliota campestris* mushroom, and of tyrosinase from *Sepia officinalis*, on tryptophan and on several of its metabolic derivatives showed that only kynurenines and some indolic compounds (5-hydroxytryptamine, 5-hydroxytryptophan and tryptamine) give a formation of black-brown pigments. The highest yield was had with 3-hydroxykynurenine.

As it was impossible with degradation to demonstrate differential features among melanins derived from tyrosine and from tryptophan and its metabolites, we have decided to follow a biogenetic route for the purpose of investigating further the role of tryptophan in melanin formation, and therefore the Harding-Passey mouse melanoma was chosen as an experimental model.

We have therefore administered D,L-tryptophan[benzene ring-^{14}C(U)] (specific activity: 95 mCi/mmol), D,L-tryptophan (methylene-^{14}C) (specific activity: 51 mCi/mmol), D,L- 5-hydroxytryptophan (methylene-^{14}C) (specific activity: 51 mCi/mmol) and 5-hydroxytryptamine-3^{1}-^{14}C (serotonin) creatinine sulphate (specific activity: 57 mCi/mmol) in mice with Harding-Passey melanoma and the incorporated radioactivity into melanin was determined after isolation from melanoma. As a comparison we have also experimented with D,L-tyrosine-2-^{14}C (specific activity: 45.4 mCi/mmol).

For each labelled compound considered, 2 groups of mice of approximately the same weight (18–22 g) were used. The mice were of Swiss albino (Arsal–Rome) strain subsequently transplanted with Harding-Passey deeply pigmented melanoma. 14 days after tumour transplanta-

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Radioactivity in Harding-Passey mouse melanoma melanin after injection of ^{14}C-labelled compounds

^{14}C-labelled compound	No. of animals	Administered radioactivity (μCi)		Tumor weight	Melanin isolated	Incorporated radioactivity (dpm)	
	treated	Per mouse	Total	total (g)	total (mg)	Total	Per mg melanin
D,L-tryptophan [benzene ring-^{14}C(U)]	11	2.5	27.5	11.5	23.4	24,470	1,046
	11	10.0	110.0	13.3	36.4	186,520	5,124
D,L-tryptophan-methylene-^{14}C	4	2.5	10.0	7.5	5.0	7,560	1,512
	4	10.0	40.0	5.9	8.0	41,584	5,198
D,L-5-hydroxytryptophan-methylene-^{14}C	4	2.5	10.0	3.9	9.1	3,600	396
	4	10.0	40.0	3.2	8.3	2,230	269
5-Hydroxytryptamine-3′-^{14}C	4	2.5	10.0	4.5	10.6	2,790	263
	4	10.0	40.0	2.5	6.9	2,640	383
D,L-tyrosine-2-^{14}C	4	2.5	10.0	2.9	7.6	9,211	1,212
	4	10.0	40.0	7.9	24.4	128,686	5,274