Thin Layer Chromatography and Rf Values of Amino Acid-Copper Complexes in Phenol/Water

Preparation of complexes. 2 volumes of amino acid solution in 10% iso-propanol (Shandon Scientific Company, London) were mixed with 1 volume of cupric Chloride solution, both at 0.01 *M*. The resulting solution was kept at room temperature until required.

Thin layer chromatography. This was performed with batches of 12 glass plates, each 20 × 20 cm and coated with Kieselgel G (Merck) to a thickness of 500 μ, held in a stainless steel frame. Samples (copper complex or free amino acid) were applied in volumes of 10 or 15 μl, 10 spots to each plate, and chromatography was performed 24 h later. The solvent was 3:1 (w/w) phenol:water. After a run of up to 4 h, the plates were placed in a 110 °C oven to remove solvent.

Free amino acids were detected with 0.2% ninhydrin in acetone. This reagent reacted also with amino acids chelated with copper. No attempt has been made to describe the colours obtained with the latter, since it is impossible to describe adequately the various shades of pinks, purples, browns and yellows, and their subtle and occasionally fundamental changes. It was also found important to view the plates always from the front as the colours given by some amino acids appeared quite different when seen from the reverse side of the plate.

Duplicate plates were sprayed with a 0.1% solution of diethyldithiocarbamate in 50% ethanol to detect copper. The metal gave a brown colour with this reagent even when chelated by an amino acid. There was no need for preliminary spraying with dilute mineral acid to release free cupric ions. None of the un-complexed amino acids gave any apparent reaction with the copper stain.

Results and discussion. All free amino acids gave single discrete spots except for the basic ones which tended to 'streak'. The copper complexes also gave single discrete spots when stained with either ninhydrin or for copper.

The complex had in every case a different Rf from its parent amino acid, and the complex when stained with the copper reagent gave the same Rf as when it was stained with ninhydrin. With the exception of the lysine-copper complex, all complexes migrated at a faster speed than their parent amino acids.

Rf of amino acids and their copper complexes in phenol/water

Amino acid	Rf of amino acid	Rf of copper complex	
		Stained with ninhydrin	Stained with 'copper' reagent
Ala	27	43	43
Arg	18	28	28
Asp	4	6	6
Glu	6	36	36
Gly	23	34	34
His	32	45	44
HO-pro	39	43	43
Ileu	51	84	83
Leu	51	80	81
Lys	11	5	5
Met	51	78	79
Phe	65	86	87
Pro	53	56	56
Ser	19	24	24
Thr	24	38	38
Try	68	85	83
Tyr	51	71	72
Val	40	51	51

Résumé. On a déterminé les Rf de 18 acides aminés et de leurs complexes de cuivre dans un système silica-phénol-eau. Dans ce milieu, les complexes ne sont pas dissociés et chaque acide aminé possède un Rf différent du Rf de son complexe de cuivre.

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The Effect of Treatment with Parasympatholytics on the Weight of the Submaxillary Gland of Rats

It has been suggested that the size of salivary glands is dependent on the secretory activity[1]. This agrees with the finding that surgical denervation by section of the parasympathetic secretory nerves causes a glandular atrophy[2]. On the other hand, while 'pharmacological' denervation by treatment with parasympatholytics does not decrease the weight of salivary glands it does cause changes similar to those seen after surgical denervation[3,4]. It was occasionally observed, however, that treatment with big doses of atropine seemed to reduce the weight of the rat's submaxillary gland. This was further investigated using atropine or an atropine-like substance, Hoechst 9980[5].

33 female rats bred at this Institute were used. The animals were 110 days old and weighed about 200 g. The right submaxillary gland was parasympathetically denervated in all animals. A preganglionic parasympathetic denervation was achieved by section of the chorda-lingual nerve. 12 rats were given atropine while 10 animals were treated with Hoechst 9980; the drugs were injected sub-

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cutaneously for 1 week. 11 rats were kept 1 week as untreated controls to study the effect of surgical denervation alone on the weight of the submaxillary gland. Atropine as the sulphate was given at intervals of 6 or 12 h and Hoechst 9980 (piperidino-ethyl-diphenyl-acetamid hydrochloride) at intervals of 6 h. The doses of the drugs were gradually increased during the period of treatment. In the group of rats treated with atropine at intervals of 12 h the dose was increased from 5–20 mg, total amount 180 mg; in the other atropine-treated group the dose was augmented from 1–5 mg, total dose 84 mg. The dose of Hoechst 9980 was increased from 0.5–2 mg in one group and from 1–5 mg in another, total amounts 36 and 84 mg respectively. All rats were killed by cervical dislocation after 1 week 4–6 h after the last injection. The submaxillary glands were carefully dissected, cleaned and weighed (wet weight). The dry weight of the glands was estimated after heating to 105–110 °C for 48 h.

Surgical parasympathetic denervation was found to decrease the dry weight of the submaxillary gland by about 25% in 1 week, from 37 ± 1.5 (11)[6] to 28 ± 1.6 (11) mg (Table). Treatment with atropine caused a similar, significant decrease in the weight of the normally innervated gland (Table). The reduction in the size of the glands can be considered to be specific in the group given 84 mg atropine but not in that given 180 mg atropine since the rats in the last group, but not in the first one, showed a marked decrease in body weight. Treatment with Hoechst 9980 had an effect similar to that of atropine on the weight of normally innervated glands when given in sufficient amounts (Table). The body weight was not affected by the doses of Hoechst 9980 used. Treatment with atropine or Hoechst 9980 did not change the weight of the parasympathetically denervated submaxillary glands. The dry weight of the glands was found to be about 25% of the wet weight in all groups.

'Pharmacological' denervation has been found to induce changes, e.g. supersensitivity to secretory agents, similar to those seen after surgical denervation in salivary glands of cats[3,7] and rats[4], while it does not cause a glandular atrophy. In the present investigation it was found that treatment with parasympatholytics, atropine or Hoechst 9980, reduces the weight of the submaxillary gland of rats. It should be recalled that the doses of atropine and particularly Hoechst 9980 are markedly bigger than those used in previous experiments. The present finding agrees with the suggestion that the size of salivary glands is dependent on the organ activity[1].

Dry weight of the right and left submaxillary gland 1 week after parasympathetic denervation of the right gland in untreated rats (= control) and in animals treated with a parasympatholytic agent, atropine or Hoechst 9980

Group	Total dose (mg)	No. of rats	Dry weight (mg) Right	Left	Right/left %
Control	–	11	28 ± 1.6	37 ± 1.5	74 ± 2.9
Atropine	84	5	28 ± 1.4	31.9 ± 0.96[b]	88 ± 2.0[b]
Atropine	180	7	26 ± 2.3	30 ± 1.8[b]	86 ± 4.5[a]
Hoechst 9980	36	5	28 ± 2.2	35 ± 2.3	82 ± 2.7
Hoechst 9980	84	5	27.5 ± 0.94	31.3 ± 0.65[b]	88 ± 1.3[c]

Values are mean ± S.E.M. [a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ when compared with the corresponding control value.

Zusammenfassung. Parasympathische chirurgische Denervierung führt zur Gewichtsabnahme der Speicheldrüsen; die Submaxillarisdrüse der Ratte zeigt eine Atrophie nach Behandlung mit parasympatholytischen Substanzen (Atropin oder Hoechst 9980).

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Action of Thalidomide on Endosteal Ossification in the Pigeon

The endosteal ossification is a phenomenon which occurs naturally in female birds, depending on the ovarian cycle[1–4].

In the pigeon, when the ovarian follicle reaches the dimension of 4–5 mm, a spicular network begins to form, which increases in a centripetal sense from the internal surface of the diaphysis of the long bones endowed with blood-forming bone marrow. When the follicle size has reached its maximum (about 20 mm in diameter) the endosteal bone has completely invaded the cavity and the marrow is reduced to islands between the spicular network. In connection with the formation of the calcareous egg-shells, there is a rapid regression of the newly-formed bone, and a few days after the deposition the initial situation is restored and the bones present a normal red bone marrow.

This cyclic phenomenon, which has the significance of realizing a deposit of Ca to be utilized in the formation of the calcareous shell, can be provoked experimentally both in the female and in the male birds by administration of estrogens[5].

I previous research[6], it was observed that pigeons (*Columba livia* Gm.) which had received estrogens and thalidomide for 20 days showed, in X-ray and in histological preparations, a poorer endosteal ossification com-

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