MAGE[8]. The precipitates were collected by microfiltration and assayed for radioactivity. The precipitate of antiserum incubated with ACTH-I^{131} was strongly radioactive whereas the precipitate of normal serum incubated in the same conditions did not show any significant radioactivity (Table).

Incubation of successive dilutions of antiserum or normal serum with a constant dilution of ACTH-I^{131} shows that the radioactivity increases with increasing concentrations of antiserum, whereas no change occurs with normal serum (Figure 2).

In the study of the binding of ACTH to its antibodies, ACTH-I^{131} was added to a suspension of the antibodies precipitated by anti-γ-globulin. The mixture was incubated at 0° and microfiltration carried out at regular intervals. The radioactivity of the precipitate collected on the microfilters increased with time, reaching a maximum after 90 min (Figure 3).

The reversibility of the process and its time dependency was verified by the decrease in radioactivity of the precipitated complex following the addition of an excess of unlabelled ACTH (Figure 4).

When unlabelled ACTH is added to ACTH-I^{131} prior to the addition of antiserum and anti-γ-globulin, the radioactivity of the resultant precipitate decreases with increasing concentrations of added unlabelled ACTH (Figure 5). The system allows the detection of as little as 100 $\mu\mu$g ACTH.

ACTH in human plasma can be directly compared with a dilution curve for pure ACTH. The values obtained in five normal subjects were 3 to 5 mμg ACTH in 0.1 ml when an antiserum to commercial ACTH was used and 10 to 25 mμg using antiserum to pure porcine A_1 ACTH. In a subject treated with large doses of prednisone (200 mg per day) the ACTH level was 0.7 mμg in 0.1 ml using the antiserum to commercial ACTH.

Discussion. Our data confirm the existence of antibodies to ACTH which were first demonstrated by haemagglutination by FISHMAN, MCGARRY and BECK[2]. Like the antibodies to insulin[9] and to glucagon[10,11], they are non-precipitating. The data also demonstrate that the binding of ACTH to its antibody is time dependent and that the process is reversible.

The decrease in radioactivity observed after addition of unlabelled ACTH to the mixture of antiserum, ACTH-I^{131} and anti-γ-globulin provides the basis for an immunoassay. The sensitivity of the assay allows the determination of the basal level of human plasma ACTH and of the ACTH level during inhibition of the pituitary release of ACTH by cortisol or its derivatives. The differences observed in the ACTH level of normal subjects when using antiserum to different ACTH preparations show that absolute values will only become possible when human ACTH is used as a standard[12].

Résumé. Des anticorps anti-ACTH ont été produits. Ils forment avec l'ACTH un complexe non précipitable. Ils ont servi au développement d'un test radio-immunologique de détermination de l'ACTH.

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Binding of ACTH-I^{131} to antibodies

		Counts per min in the precipitate
ACTH-I^{131}	+ antiserum 1/100	226
	+ normal serum 1/100	25
	+ phosphate buffer	27

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Catecholamines of the Spinal Cord Normally and after Transection

Using a new histochemical technique, CARLSSON et al.[1] have obtained strong evidence for the view that the noradrenaline present in the central nervous system serves as a neurotransmitter. Other data supporting this view are presented in this paper.

The experiments included five rabbits, the spinal cords of which were cut at the level of the 2nd thoracic segment under ether anaesthesia. The transection was performed with a pair of scissors after an incision in the median line of the back. The animals were given a suspension of penicillin and streptomycin immediately after the operation. They seemed to feel well and to take their food normally, and were killed 6–7 days after the operation. The noradrenaline contents of the parts of the cord above and below the transverse section were determined using the methods described by BERTLER, CARLSSON and ROSENGREN[2] and HÄGGENDAL[3]. Control values were obtained from animals not operated on. The values are found in the Table.

It will appear from the Table that the spinal cord of rabbit contains 0.15 μg noradrenaline per g. Its content of

Noradrenaline content of the spinal cord of the normal rabbit and after transection at the 2nd thoracic segment. The figures indicate μg/g

Controls		Operated animals	
above Th 2	below Th 2	above Th 2	below Th 2
0.13	0.12	0.14	0.02
0.15	0.15	0.10	0.02
0.11	0.11	0.12	0.03
0.09	0.10	0.09	0.03
0.29	0.26	0.11	0.01

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this amine is thus about one third of the concentration in the brain. It is also seen that after the cross section the noradrenaline of the part caudal to the section disappears almost completely. This fact seems to us to indicate that the major part of the noradrenaline of the cord is located in nerve fibres, the cell bodies of which occur in an area of the central nervous system rostral to the section. This is taken as an additional proof for the view that the noradrenaline of the central nervous system is a neurohormone. Work is in progress in order to localize the cell bodies.

In contrast to other investigators, we have not been able to detect any significant amount of dopamine in the spinal cord of the rabbit or the rat. The values obtained by us were 0.01 µg/g or less, i.e. only about one hundredth of the amount found by McGEER and McGEER[4,5].

Zusammenfassung. Der Noradrenalingehalt im Rückenmark des Kaninchens beträgt 0.15 µg/g. Eine Querschnittsläsion durch das Rückenmark führt zu einer 90%igen Abnahme des Noradrenalingehaltes. Dies legt die Annahme nahe, dass Noradrenalin in Nervenzellen des Zentralnervensystems lokalisiert ist. Im Gegensatz zu anderen Untersuchern fanden wir im Rückenmark kein Dopamin.

T. MAGNUSSON and E. ROSENGREN

Department of Pharmacology, University of Gothenburg (Sweden), January 8, 1963.

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Masking Action of Basic Proteins on Sialic Acid Carboxyls in Epithelial Mucins[1]

Histochemical studies on polysaccharides of different mucous-producing glands have clearly established that tissue basophilia, revealed with different cationic dyes (alcian blue, colloidal iron, azure A), is due to the presence of sialic acid (SPICER and WARREN[2], QUINTARELLI et al.[3]). Chemical investigations on salivary mucins further demonstrated that sialic acid occupies terminal positions and that mild acid hydrolysis releases sialic acid without further cleavage of the mucin molecule (GOTTSCHALK[4]).

Accordingly, when the mild hydrolytic treatment was applied to submaxillary gland sections it was found that in some animals basophilia was completely removed while in other animals similar results were obtained only when stronger acid solutions, higher temperatures and longer incubation times were applied (QUINTARELLI et al.[5]). Furthermore, the use of the specific enzyme neuraminidase demonstrated that whereas in some tissues sialic acid was easily split off from the glycoprotein molecule, in other animal glands neuraminidase had only a slight hydrolytic effect (QUINTARELLI et al.[5], WARREN and SPICER[6]). These findings suggested that, although most of the salivary mucins contained sialic acid, their structural configuration varied widely from one animal gland to another.

Recent chemical and histochemical studies on several mammal salivary mucins have disclosed that sialo-glycoproteins are present not only in the mucous-producing glands but also in the parotid (AURELI et al.[7]). Furthermore, treatment of parotid and submaxillary sections with proteolytic enzymes (pepsin, crude and crystalline papain) gave rise to opposite and quite unexpected results. While in some mammals proteolysis destroyed submaxillary mucins, thus leaving no substrate to be stained in the section, in other animal glands the staining for acid polysaccharides was greatly augmented after proteolytic treatment. These latter findings suggested that the sialic acid carboxyls responsible for the dye-binding were somewhat blocked by basic proteins (QUINTARELLI[8]), probably in the same manner as some acid mucopolysaccharide-protein complexes in the connective tissue ground substance (KELLY[9], FRENCH and BENDITT[10], QUINTARELLI[11]).

The results reported heretofore seemed to indicate that in some animal mucins sialic acid may be found in two different forms: an unbound form, susceptible to mild acid hydrolysis, and a bound one which could only be revealed after the action of proteolytic enzymes. Since these findings needed further examination, additional histochemical procedures were used in an effort to assess more data which could either substantiate or contradict the original results.

Submaxillary glands of monkeys (*Macaca mulatta*) were utilized together with dog parotids. Fresh tissues were fixed in alcohol-formaldehyde, dehydrated, cleared and embedded in the usual fashion. Deparaffinized sections were immersed in a 0.1 *N* Na-acetate/HCl solution, pH 2.5 at 75° for 1, $1^1/_2$ and 2 h. Control sections were incubated in distilled water at the same temperature and for the same lengths of time. After treatment, sections were rinsed in distilled water, dried and then incubated in pepsin or crude papain for 2 h. Papain was activated by incubating the enzyme at 37° for 30 min in 20 ml of 0.02 *M* acetate buffer, pH 5.4, which contained KCN and E.D.T.A. in a concentration of 5 µm. Pepsin was prepared in a 0.02 *N* Na-acetate/HCl solution, pH 2.5 containing

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