tion of serum. The rats were sacrificed 50 h after hepatectomy (26 h after the injection of serum). Paraffin sections were made, stained with hematoxylin-eosin, and the mitoses were then counted in 100 fields (5000 cells). The mitotic index (stathmocinetic index) was expressed as the number of mitoses per 100 cells.

From the results of our experiments (Table), it may be assumed that intraperitoneal injections of serum from partially hepatectomized rats have a stimulating effect on mitosis in the regenerating livers of all animals. The rise in the mitotic index was, however, lower in all those cases where the donor rats had been irradiated.

It may be preliminarily concluded that radiation reduces the mitosis-stimulating activity of serum. It is quite possible that the amount of a mitosis-stimulating agent is reduced by radiation, or that the amount of a mitosis-inhibiting agent—whether specific or non-specific—is increased as a result of radiation.

Zusammenfassung. Der Mitoseindex in der Rattenleber wird durch Injektion von Serum hepatektomierter Ratten beträchtlich gesteigert. Werden die Donatortiere mit 700 r bestrahlt, dann ist dieser Effekt signifikant reduziert.

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ACTH Antibodies and their Use for a Radioimmunoassay for ACTH

Until recently no conclusive evidence of specific antibodies to ACTH has been obtained because of the lack of precipitation of the ACTH-antibody complex[1]. The first demonstration for the antigenicity of ACTH was presented with the use of an haemagglutination technique[2,3]. Using a modification of the Yalow and Berson competitive inhibition technique[4] further evidence is presented for the antigenicity of ACTH. Details of the binding between ACTH and antibody are given.

The development of a sensitive method for the determination of circulating ACTH has long been awaited. The immunoassay presented here is based on a modification by Hales and Randle[5] of the insulin radioimmunoassay of Yalow and Berson[4]. It allows the detection of as little as 100 μμg ACTH in 0.1 ml and makes possible the determination of ACTH in normal human plasma as well as in plasma of patients whose ACTH level has been lowered by large doses of cortisol or derivatives.

Material and Methods. ACTH: Pure porcine A_1 ACTH was a gift from Dr. H. B. F. Dixon of the Department of Biochemistry of the University of Cambridge. It was obtained by a modification of the method of Dixon and Stack-Dunne[6] and found to be chromatographically pure.

ACTH-I^{131}: ACTH was labelled with I^{131} by an adaptation of the method developed by Hales and Randle[5] for insulin (on the principle given by McFarlane[7]). 50 μg ACTH in 10 μl pH 1.8 glycine-HCl buffer was incubated for 5 min with a mixture of 20 μl ICl and 4 to 12 mC NaI^{131} in 50 μl. It was then dialysed for 24 h against five changes of 0.01 *N* NH_4OH and purified on a cellulose column[4] using 15% human albumin as eluant. The specific activity obtained varied between 4 and 10 mC per mg ACTH.

Antibodies: One guinea-pig was immunized with pure porcine ACTH (7 mg in 5 injections) and two guinea-pigs with a commercial preparation of ACTH (75 international units in 5 injections). The animals were not adrenalectomized. For immunization these hormones were emulsified in complete Freund's adjuvant.

Anti-γ-globulin: Rabbit anti-guinea-pig γ-globulin was prepared by injecting rabbits with a preparation of crude guinea-pig γ-globulin emulsified with complete Freund's adjuvant. The guinea-pig γ-globulin was obtained by precipitation of 2 volumes of normal guinea-pig serum with one volume of saturated $(NH_4)_2SO_4$.

Buffers: Dilutions were made in 0.04 *M* phosphate buffer pH 7.4 containing 1/4000 merthiolate and 2 mg per ml

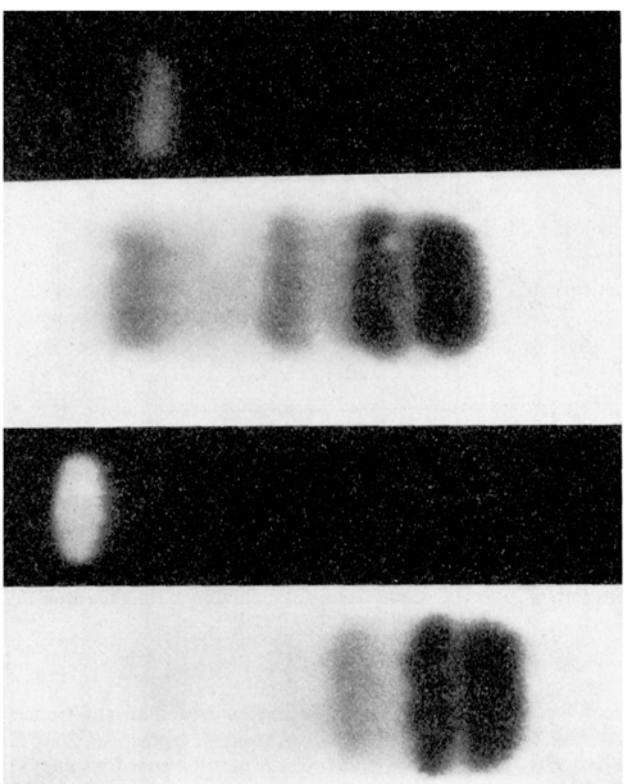

Fig. 1. Paper electrophoresis of antiserum (above) and normal serum (below) incubated for 24 h at 0° with ACTH-I^{131}. The electrophoresis was done in veronal buffer pH 8.6, 0.1 ionic strength at +4° for 15 h at constant voltage 4 V per cm. The main peak of radioactivity shown by contact photography moved with the γ-globulins in the incubation of the antiserum, whereas it remained at the origin in the case of normal serum.

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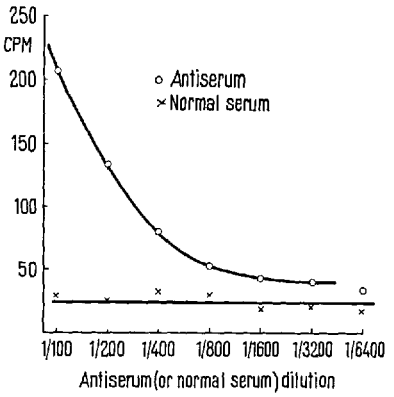

Fig. 2. Antibody dilution curve. Successive dilutions of antiserum or normal serum in 0.1 ml were incubated at +4° with 0.1 ml of a constant dilution of ACTH-I^{131}. The antiserum (or normal serum) dilution is plotted against the radioactivity in counts per min of the precipitate.

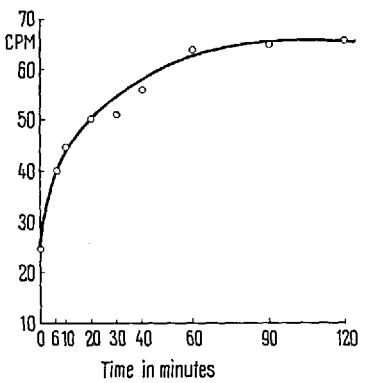

Fig. 3. Binding of ACTH-I^{131} to antibodies. 0.1 ml ACTH-I^{131} was added at time 0 to 0.1 ml of a suspension of antibodies precipitated by anti-γ-globulin. The incubation was carried out at 0° and the microfiltration done at regular intervals. The radioactivity of the precipitate collected on the filter is plotted against the time after the addition of ACTH-I^{131}.

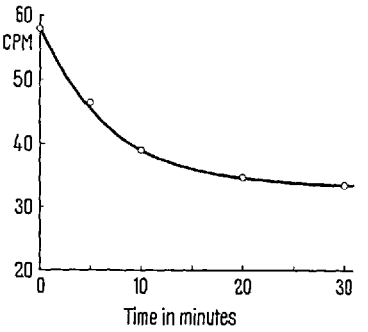

Fig. 4. Dissociation of ACTH-I^{131} from the ACTH-I^{131} antibody complex. 5 µg unlabelled ACTH in 1 µl were added 60 min after the addition of 0.1 ml ACTH-I^{131} to 0.2 ml of a suspension of antibodies precipitated by anti-γ-globulins. Microfiltration of the suspension was carried out at regular intervals. The radioactivity of the precipitate collected on the microfilter is plotted against the time after the addition of the unlabelled ACTH.

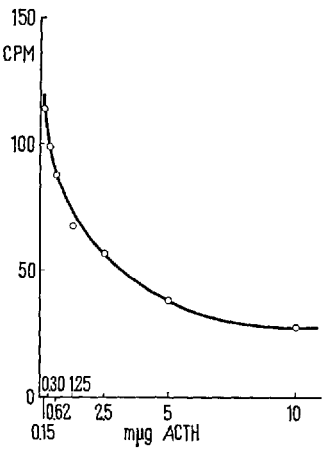

Fig. 5. Radioactivity of the precipitate as a function of added unlabelled ACTH. Increasing concentrations of ACTH in 0.1 ml was incubated with 0.1 ml ACTH-I^{131} and 0.1 ml antiserum 1/100. The radioactivity of the precipitate is plotted against the concentration of added unlabelled ACTH.

beef serum albumin. 0.9% NaCl was added to the buffer for the dilutions used in the standard curves of ACTH when the curves were to serve as a reference for plasma ACTH.

Incubations and Assay: In the study of the binding of ACTH-I^{131} to antibody and of the dissociation of the ACTH-I^{131}-antibody complex by excess unlabelled ACTH, one volume of antiserum diluted 1/100 was first mixed with two volumes of anti-γ-globulin diluted 1/5. 0.2 ml of the mixture was incubated for 24 h in a series of small test tubes. On the following day 0.1 ml of diluted ACTH-I^{131} was added to every test tube, mixed well with a vibrating mixer and the precipitates collected by microfiltration at regular intervals. The dissociation of the ACTH-I^{131}-antibody complex was obtained by addition of 5 µg unlabelled ACTH in 1 µl. The whole process was carried out at 0°.

In the antibody concentration curves or in the ACTH assay, 0.1 ml of varying concentrations of unlabelled ACTH or 0.1 ml plasma or buffer was incubated with 0.1 ml diluted ACTH-I^{131} and 0.1 ml diluted antiserum. After 4 h incubation at +4°, 0.1 ml anti-γ-globulin diluted 5 times was added. The incubation was continued at +4° for 16 to 20 additional hours. The precipitates were then collected on Oxoid filter membranes using a Millipore filter holder. They were washed on the membrane with 0.04 *M* phosphate buffer pH 7.4 containing 1/4000 merthiolate and 50 mg/ml beef serum albumin and assayed for radioactivity in a Model D47 nuclear gas flow counter with automatic sample changer.

Human blood was drawn into a syringe previously moistened with heparin and immediately centrifuged at +4°. The plasma was separated into a test tube which was sealed and kept frozen until required for the ACTH assay.

Paper electrophoresis: Paper electrophoresis was performed in veronal buffer, 0.1 ionic strength, pH 8.6, at +4° for 15 h at constant voltage 4 V/cm.

Results. Paper electrophoresis in veronal buffer pH 8.6 of ACTH-I^{131} previously incubated for 24 h at 0° with anti-serum or with normal serum showed that ACTH-I^{131} does not migrate when incubated with normal serum but moves with the γ-globulin when incubated with antiserum (Figure 1).

As the ACTH-antibody complex does not spontaneously precipitate, it was precipitated with rabbit anti-guinea-pig γ-globulin according to the technique of SKOM and TAL-

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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