la connaissance des systèmes complexes revêt une importance capitale, l'introduction de concepts élaborés dans un secteur peut, plus qu'hier, éclairer la progression du savoir. La constitution d'équipes de chercheurs aux fonctions diverses, s'enrichissant les uns et les autres des fruits de démarches différentes, est une nécessité. Mais la solidité de telles équipes est compromise par leur spécialisation bien trop précoce dans les cursus de formation, généralement basés sur une pulvérisation de la connaissance, liée à une exacerbation de la méthode analytique.

Enfin, au stade de l'application, la gestion et l'aménagement de l'espace sont assurés par des personnes à qui leur formation permet d'avoir seulement une vision partielle du problème, généralement limitée aux aspects économiques ou technologiques.

Si l'accroissement continu des connaissances exige toujours la formation de spécialistes, il faut parallèlement former des généralistes, ayant une formation totale tout aussi importante, mais dont la connaissance au lieu de se limiter à un secteur particulier, s'étend à l'ensemble du domaine concerné.

L'accroissement du savoir humain réhabilite la notion de généraliste comme complément indispensable des spécialistes. Un nouvel humaniste du XXème siècle associant sciences de la nature et sciences humaines doit être développé d'extrême urgence.

La révolution scientifique et technique entraîne une révolution dans l'approche des problèmes, l'introduction d'une conception écologique. Cette introduction n'aura que des effets limités, insuffisants, sans rapport avec l'importance et l'urgence des problèmes, si elle n'est pas accompagnée d'une refonte totale dans les processus de formation hérités du XIXème siècle.

Summary. With the increased impact of man's activities resulting from the industrial revolution, the fall-out of his actions widens in both space and time. It is impossible to proceed any further in the exploitation of Nature on the basis of economic concepts founded on the pursuit of sectorial short-term profit. We must on the contrary observe the rules governing the biosphere and the ecosystems.

Economic development must not upset the energetic equilibrium of the biosphere. This is possible on condition that we use essentially balanced energy sources, instead of the energy released by potential sources.

The whole organization of economy is based on a linear principle, resulting in the exhaustion of basic materials and overload by often polluting waste. The acceleration of the circuit is artificially induced by publicity factors which, pretexting fashion, regards as garbage still usable goods. The perenniality of ecosystems is ensured by the recycling of materials, an economy founded on the same principle can alone ensure the future of mankind.

The sectorial development of economy and of professional training contributes to make it more difficult to organize the healthy management of natural resources.

The obstacles to the achievement of a healthy policy of resource management are not technical, but pedagogic, economic and political.

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PRO EXPERIMENTIS

Radioimmunoassay of Free Aldosterone and of its 18-Oxo-Glucuronide in Human Urine

So far, only a few reports have been published on the estimation of free aldosterone in urine, because various methodological difficulties have prevented its measurement. For this reason, the following questions cannot be answered satisfactorily: 1. Is the amount of free aldosterone in urine of diagnostic usefulness? 2. Is the excretion of aldosterone influenced by alterations of renal function? and 3. What is the relationship between free aldosterone in urine and aldosterone plasma levels, aldosterone secretion rate, or the excretion of the acid labile metabolite, 18-oxo-glucuronide? Recently, radioimmunoassays for the determination of aldosterone in plasma and of the acid labile metabolite in the urine have been published[1–3]. The present report describes a radioimmunological method for the determination of free aldosterone and of the acid labile metabolite in urine.

Method. New Zealand white rabbits were immunized with a complex of D-aldosterone-21-hemisuccinate and bovine serum albumin, prepared according to the procedure described by ERLANGER et al.[4]. 1 mg of the antigen was suspended in 0.5 ml 0.9% saline solution and 0.5 ml of complete Freund's adjuvant (DIFCO) immediately before the intramuscular injection. Similar injections were given every 2 to 4 weeks. After 6 monts, 5 of the 6 immunized rabbits had antibody titers of 1:8000 to 1:12000 (Table I and Figure 1).

Cross reactions of the antiserum with several steroids are given in Table II. The degree of cross reactions was calculated according to the method of ABRAHAM[5]. The results obtained are apparently more specific than those described by KELLY[6], who also used as antigen an aldosterone-21-hemisuccinate-bovine albumin complex. However, the cross reactions observed with our antiserum were similar to those reported by MAYES et al.[1], who used as antigen an aldosterone-3-oxime-bovine serum albumin complex.

The free aldosterone and the acid labile metabolite were extracted from urine and separated by a modification of the method described by MAYES et al.[1]. (Table III). The most important difference between the methods was the use of 2 different paper chromatography systems for the separation of aldosterone: Bush B 5 and isoamylacetate/water[7]. Since the Rf-value of D-aldosterone as compared with that to other steroids of similar polarity in the iso-

[1] D. MAYES, S. FURUYAMA, D. C. KEM and C. A. NUGENT, J. clin. Endocrin. *30*, 682 (1970).
[2] F. BAYARD, I. Z. BEITINS, A. KOWARSKI and C. J. MIGEON, J. clin. Endocrin. *31*, 1 (1970).
[3] F. BAYARD, I. Z. BEITINS, A. KOWARSKI and C. J. MIGEON, J. clin. Endocrin. *31*, 507 (1970).
[4] B. ERLANGER, F. BOREK, S. M. BEISER and S. J. LIEBERMAN, Biol. Chem. *228*, 713 (1957).
[5] G. E. ABRAHAM, J. clin. Endocrin. *29*, 866 (1969).
[6] W. KELLY, in *Immunologic Methods in Steroid Determination* (Eds. F. G. PERON, B. V. CALDWELL; Meredith, N.Y. 1970), p. 54.
[7] P. VECSEI and H. KESSLER, Experientia *26*, 1015 (1970).

amylacetate/water system differs from that in the Bush B 5 system, the purity and identity of aldosterone could be confirmed in each determination. Such a confirmation is necessary, because it cannot be excluded that unknown steroid fractions present in urine may interfere with the immune reactions.

In Figure 2 some of the values measured for free aldosterone and the acid labile metabolite in urine are summarized. The urine samples were collected from patients who stayed in bed and had a normal sodium intake. Our normal 18-oxo-glucuronide values were found to be lower than those published by LARAGH et al.[10]: 5–25 µg/day, and

Table I. Radioimmunoassay

1. 50–100 µl of the alcohol fraction containing aldosterone and 3000–6000 cpm 1-2-H^3-aldosterone were dried in polystyrene tubes[a].

2. Addition of 0.5 ml diluted immunserum. (Dilution fluid: 0.05 *M* pH 8 botate buffer, containing 0.6% serum globulin[8].) Shaking for 10 min, time of incubation: 24 h.

3. Addition of 100–150 µl dextran-coated charcoal[9]. Centrifugation (1×10^4 *g*, for 15 min).

4. 0.4 ml of the supernatant were put into scintillation vials and 10 ml of Bray-scintillator were added. Measurement of the radioactivity.

Steps 2–3 at 0–4 °C.

[a] The radioactivity in the fraction 50–100 µl at the end of the isolation procedure was in most cases negligible (less than 50 cpm). Where this not the case, the observed radioactivity has to be corrected up to the radioactivity used for the calibration curve (3000–6000 cpm).

Table II. Cross reactions of different steroids with the aldosterone antibodies

	Rabbit No. 7 (%)	Rabbit No. 8 (%)	Rabbit No. 9 (%)
Aldosterone	100	100	100
Aldosterone-21-Acetate			100
Corticosterone	3.4	1.69	7.6
Desoxycorticosterone	0.12	0.12	1.04
Progesterone	0.10	0.13	1.16
Cortisol	0.37	0.08	0.48
Cortison	0.16		0.27
18-OH-Corticosterone	0.24	0.18	0.05
18-OH-Desoxycorticosterone			0.48
11-OH-Progesterone	0	0.05	0.29
17β-Oestradiol			0
21-OH-Pregnenolone			0

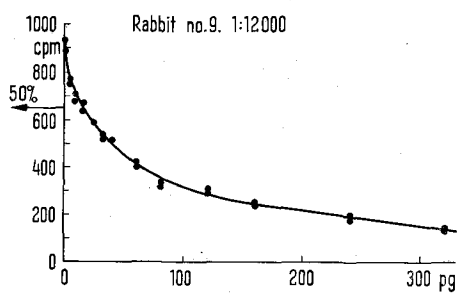

Fig. 1. Calibration curve-obtained with different amounts of unlabelled aldosterone. Time of incubation: 24 h at 2 °C. Ordinate: bound aldosterone radioactivity (cpm). Abscissa: different amounts of aldosterone (pg). Blank value: eluate of chromatographic paper without steroid = 0 pg.

Table III.

a) Isolation of free aldosterone

1. 100–300 ml urine
   ↓ addition of 5000–12 000 cpm 1-2-H^3-aldosterone[a] (125 cpm = 1 pg)

2. Extraction with dichloromethane and purification (0.1 NaOH + H_2O).

3. Chromatography ($^1/_2$ vol. in Bush B 5, $^1/_2$ vol. in isoamylacetate/water system; Whatman No. 1., 3 cm).

4. Localization of aldosterone by means of a parallel strip with unlabelled aldosterone.

5. Elution and microcolumn chromatography (Silicagel 70–325 mesh, 5 ml ethylalcohol).

6. 50–100 µl of the total alcohol fraction (5 ml) for the radioimmunoassay, 1 ml for measuring of radioactivity.

b) Isolation of the acid labile metabolite

20–40 ml urine
↓
1. Extraction with dichloromethane (removal of the free steroids), pH 1 for 18–24 h.
2. ↓ addition of 5000–12 000 cpm 1-2-H^3-aldosterone,
3.–6. as steps 2–5 for the separation of free aldosterone,
7. after dilution (1:4 to 1:8), 50 µl are taken for the radioimmunoassay, and 1 ml for the measuring of radioactivity.

[a] In some experiments 30–60 000 cpm 1-2-H^3-aldosterone was added to the sample. In those experiments the radioactive aldosterone was localized by means of a radiochromatogram scanner.

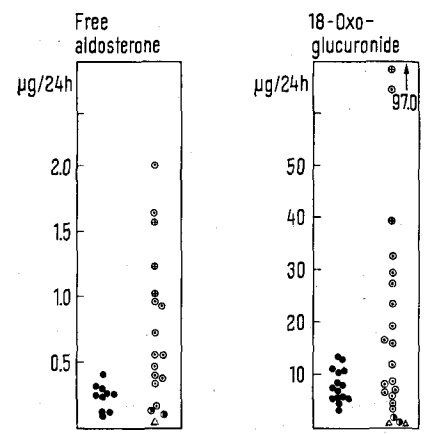

Fig. 2. Excretion of free aldosterone and of aldosterone 18-oxo-glucuronide in healthy subjects (full circles, left side of the column) and in patients with various disorders: hypertension with suspect of aldosteronism (free aldosterone: 12 cases; 18-oxo-glucuronide: 15 cases). ○, Conn-syndrome due to solitary adenoma (one case); ⊕, preoperative; ◐, postoperative, △, adrenal insufficiency (2 cases).

by NEW et al.[11]: 5–20 µg/day. However, our results are similar to the recent data of AAKVAAG[12].

By using the prodecure described, 20–25 measurements of free urinary aldosterone or 15 measurements of the acid labile metabolite per week can be done by an experienced technician.

Zusammenfassung. Von Kaninchen, die mit einem D-Aldosteron-21-hemisuccinat-Rinderserumalbumin-Komplex immunisiert waren, konnten Aldosteron-Antikörper von ausreichendem Titer und von guter Spezifizität gewonnen werden. Mit Hilfe dieser Antikörper war es möglich, freies Aldosteron und den 18-oxo-Glucuronid-Metaboliten im Urin von gesunden Versuchspersonen und bei Patienten mit veränderter Produktion von Aldosteron zu bestimmen.

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Department of Pharmacology, University of Heidelberg, Hauptstrasse 47–51, D-69 Heidelberg (Germany),
10 November 1971.

[8] *Human Serum Globulin: Forschungs-Gamma-Globulin* (Deutsche Kabi GmbH, München).

[9] 1 vol. activated coal solution (5 g Norit A, Serva, Heidelberg, in 80 ml γ-globulin buffer solution) +3 vol. Dextran solution (100 mg Dextran T 70), Pharmacia, Uppsala, dissolved in 80 ml γ-globulin-buffer solution. Stirring for 10 min prior to the use.

[10] J. H. LARAGH, J. E. SEALEY and S. C. SOMMER, Circulation Res. *18–19*, 158 (1966).

[11] M. I. NEW, B. MILLER and R. E. PETERSON, J. clin. Invest. *45*, 412 (1966).

[12] A. AAKVAAG, Clin. chim. Acta *34*, 197 (1971).

[13] Present address: Department of Organic Chemistry of the University Szeged (Hungary).

Corrigendum

K. K. MÄKINEN: *Activation of Subtilisin and Luteinizing Hormone*, Experientia *27*, p. 1261 (1971). In the legend to the figure on page 1262 on the 4th and 5th line the word luteinizing should correctly be **luteotropic.**

CONGRESSUS

USA

3rd Congress of the International Society on Thrombosis and Haemostasis, in conjunction with the Council on Thrombosis, American Heart Association

in Washington, D.C., 22–26 August 1972.

The Congress will be held at the Mayflower Hotel in Washington. The topics for the planary sessions include the following: Control mechanisms in hemostasis. Cell membranes: structure and function; platelets. Molecular biology and pathophysiology of fibrinogen. Vessel wall and thrombogenesis.

Further information by Dr. Harold R. Roberts, Chairman of the Organizing Committee, Box 630, Chapel Hill, N.C. 27514, USA.

CONSTRUCTIONES

European Training Awards in Brain and Behaviour Research

In cooperation with the Organization for Economic Cooperation and Development, a group of European Scientists have initiated an experimental schema under which younger scientists working on Brain and Behaviour can apply for awards to enable them to acquire training in a specialized area. The money to finance this training program has been provided by the Max-Planck-Gesellschaft. Successful applicants will receive travel and living expenses to enable them to study in selected laboratories. The normal duration of an award will be three months, but some longer term awards can be made.

Eligibility. To be eligible for an award, a candidate must already by undertaking research in the field of Brain or Behaviour in a laboratory situated in a member country of O.E.C.D. Applicants must produce evidence that their own research will benefit by the training for which they apply. In making the awards, preference will be given to candidates applying for a type of training that will assist them to follow an interdisciplinary approach in their own research. Candidates are expected to return to their original laboratory at the expiry of their training.

Nature of training courses. Some of the training programs incorporate formal course work, others involve the learning of techniques whilst undertaking closely supervised research on a particular problem. Training programs exist in the following subjects: Animal behaviour, brain biochemistry, brain modelling, ethology, experimental psychology, histochemistry, morphology, neuroanatomy, neuropharmacology, neurophysiology etc.

Method of application. Further details of the scheme (including a list of laboratories participating in the training programs) and application forms can be obtained from:

The Executive Office, Foundation FUNGO, Laan van Meerdervoort 53D, Den Haag (The Netherlands).