It only remains to consider the conditions at the "outer" boundaries of the regions. The term "outer" boundary includes mathematical boundaries, e.g. at infinity, or the central point of a spherical region, or the axis of a cylindrical region. Let $\mathbf{r}$ and $\mathbf{r}_0$ be the radius vectors of general points within and on the "outer" boundaries of the system respectively. Then, either with reference to region *1* or region *2*, i.e. to c_1 or c_2, if c' is finite when $\mathbf{r} \to \mathbf{r}_0$, then

$$c = \frac{m}{c'_0} \int_0^t c' \mathrm{d}t$$

will also be finite as $\mathbf{r} \to \mathbf{r}_0$. Alternatively if $\partial c'/\partial n \to 0$ as $\mathbf{r} \to \mathbf{r}_0$ then

$$\frac{\partial c}{\partial n} = \frac{m}{c'_0} \int_0^t \frac{\partial c'}{\partial n} \mathrm{d}t \to 0 \quad \text{as} \quad \mathbf{r} \to \mathbf{r}_0,$$

n being the normal to the "outer" boundary.

Finally an illustration is given of the application of this transformation to linear diffusion in the normal direction across a plane interface. Consider the diffusing substance possessing the original concentration c'_0 in region *1* ($0 < x < l$) and zero in region *2* ($x > l$) and then undergoing diffusion into region *2*. The differential equations

$$\frac{\partial c'_1}{\partial t} = D_1 \frac{\partial^2 c'_0}{\partial x^2} \quad \text{for} \quad 0 < x < l,$$
$$\frac{\partial c'_2}{\partial t} = D_2 \frac{\partial^2 c'_2}{\partial x^2} \quad \text{for} \quad x > l$$

apply, with initial conditions

$$c'_1 = c'_0,\ c'_2 = 0 \quad \text{at} \quad t = 0,$$

and boundary conditions

$$\partial c'_1/\partial x = 0 \quad \text{at} \quad x = 0,$$
$$c'_1 = \alpha c'_2, \quad D_1 \partial c'_1/\partial x = D_2 \partial c'_2/\partial x \quad \text{at} \quad x = l,$$
$$c'_2 \text{ finite as } x \to \infty.$$

The solutions are

$$c'_1 = \frac{2}{\pi} c'_0 \alpha \nu \int_0^\infty \frac{\exp(-D_1 t u^2) \cdot \sin l u \cdot \cos x u \cdot \mathrm{d}u}{u(\cos^2 l u + \alpha^2 \nu^2 \sin^2 l u)}$$

$$c'_2 = \frac{2}{\pi} c'_0 \nu \int_0^\infty \frac{\exp(-D_1 t u^2) \cdot \sin l u \cdot f(u) \cdot \mathrm{d}u}{u}$$

where

$$f(u) = \frac{[\cos l u \cdot \cos\{(x-l)\nu u\} - \alpha \nu \sin l u \cdot \sin\{(x-l)\nu u\}]}{(\cos^2 l u + \alpha^2 \nu^2 \sin^2 l u)}$$

and $\nu = (D_1/D_2)^{\frac{1}{2}}$.

Application of the transformations gives

$$c_1 = \frac{2 m \alpha \nu}{\pi D_1} \int_0^\infty \frac{\{1 - \exp(-D_1 t u^2)\} \cdot \sin l u \cdot \cos x u \cdot \mathrm{d}u}{u^3 (\cos^2 l u + \alpha^2 \nu^2 \sin^2 l u)}$$

$$c_2 = \frac{2 m \nu}{\pi D_1} \int_0^\infty \frac{\{1 - \exp(-D_1 t u^2)\} \cdot \sin l u \cdot f(u) \cdot \mathrm{d}u}{u^3},$$

which form the solutions to the problem in which the diffusing substance is absent initially in both regions and is then produced uniformly throughout region *1* at rate m and diffuses into region *2*. The functions c_1 and c_2 form the solutions of the differential equations.

$$\frac{\partial c_1}{\partial t} = D_1 \frac{\partial^2 c_1}{\partial x^2} + m \quad \text{for} \quad 0 < x < l,$$
$$\frac{\partial c_2}{\partial t} = D_2 \frac{\partial^2 c_2}{\partial x^2} \quad \text{for} \quad x > l,$$

with initial conditions $c_1 = 0$, $c_2 = 0$ at $t = 0$, and boundary conditions for c_1 and c_2 which are identical with those for c'_1 and c'_2.

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Zusammenfassung

Eine grosse Anzahl von Auflösungen für mathematische Gleichungen, die sich mit der Diffusion einer Substanz von einem Raum (Raum *1*), in dem sie ursprünglich in einheitlicher Konzentration vorhanden sind, in einen anderen Raum (Raum *2*) befassen, sind allgemein zugänglich. Es wird eine Methode beschrieben, die Auflösungen dieser Art in solche umwandelt, die sich unter der Bedingung verwenden lassen, dass die diffundierende Substanz ursprünglich abwesend ist, später jedoch mit konstanter Geschwindigkeit (in Raum *1*) produziert wird. Eine solche Methode der Umwandlung ist für eine Anzahl von Grenzschichtbedingungen (boundary conditions) anwendbar.

Isolation of Crystalline Aldosterone from the Urine of a Nephrotic Patient

Since 1950 one of the present authors (J.A.L.) and his collaborators have demonstrated the occurrence of a strongly sodium-retaining corticoid fraction in the urine of normal humans and especially of oedematous patients with nephrosis or congestive heart failure[1]. The active material, originally measured by bio-assay[2], was shown to move in ZAFFARONI's paper-chromatographic system with cortisone[3], behaving thus and in other respects[4] like the highly active mineralocorticoid from adrenal cortical extracts of SIMPSON and TAIT[5]. Similar activity in urine fractions of such patients has been observed by SINGER *et al.*[6], and recently by COPE *et al.*[7] who also

[1] Q. B. DEMING and J. A. LUETSCHER, Jr., Proc. Exper. Biol. Med. *73*, 171 (1950). – J. A. LUETSCHER, Jr., Q. B. DEMING, and B. B. JOHNSON, Ciba Found. Colloq. Endocrinol. *4*, 530 (1952). – J. A. LUETSCHER, Jr., and B. J. AXELRAD, J. Clin. Endocrinol. *14*, 1086 (1954).

[2] Q. B. DEMING and J. A. LUETSCHER, Jr., Proc. Soc. Exper. Biol. Med. *73*, 171 (1950). – J. A. LUETSCHER, Jr., and Q. B. DEMING, Transactions 2nd Conf. Renal Function, J. Macy Jr. Found., 155 (1951), New York. – B. B. JOHNSON, Endocrinol. *54*, 196 (1954).

[3] J. A. LUETSCHER, Jr., and B. B. JOHNSON, Amer. J. Med. *15*, 417 (1953); J. Clin. Invest. *32*, 585 (1953). – J. A. LUETSCHER, Jr., and B. J. AXELRAD, J. Clin. Endocrinol. *14*, 1086 (1954).

[4] J. A. LUETSCHER, Jr., and B. B. JOHNSON, J. Clin. Invest. *33*, 276 (1954).

[5] S. A. SIMPSON and J. F. TAIT, Endocrinol. *50*, 150 (1952) and later publications.

[6] B. SINGER and E. H. VENNING, Endocrinol. *52*, 623 (1953). – B. SINGER and J. WENER, Amer. Heart J. *45*, 795 (1953). – M. F. MCCALL and B. SINGER, J. Clin. Endocrinol. *13*, 1157 (1953).

[7] C. L. COPE and J. GARCIA, Brit. Med. J. *1954*, 1290.

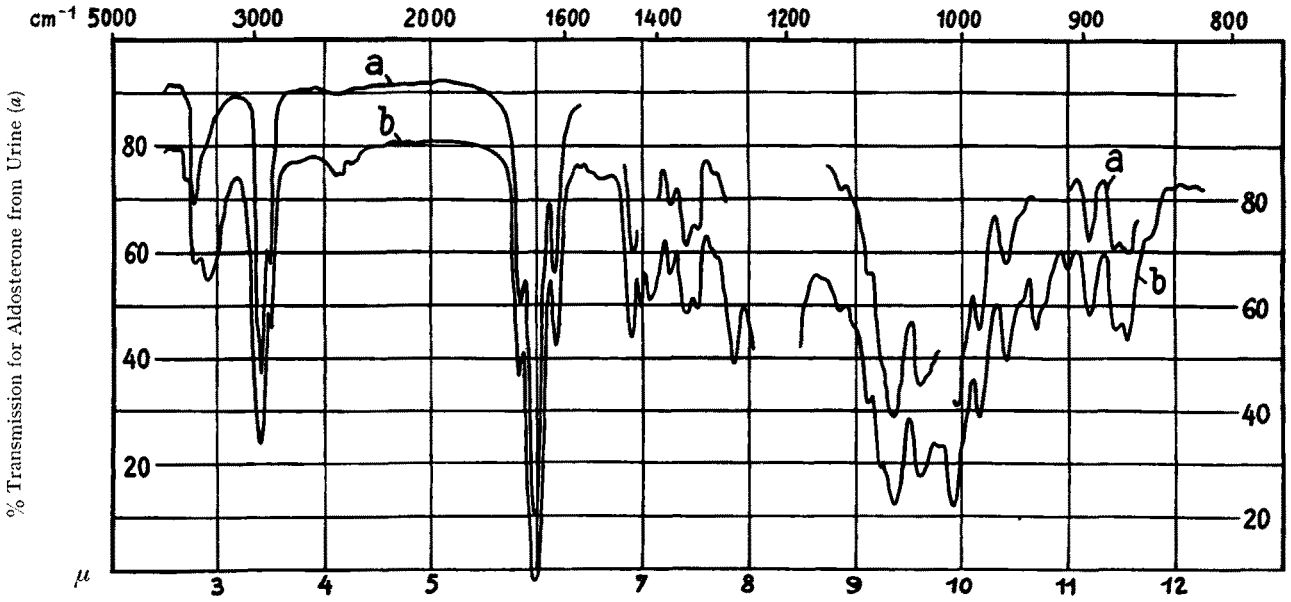

Fig. 1.–IR-Spectra of Aldosterone from Urine (*a*) and from Adrenals (*b*)[1]

[1] Determined in chloroform solution, compensated with pure solvent. Aldosterone from urine: ca. 0·02 molar, thickness 1·0 mm. Aldosterone from adrenals: 0·10 molar, thickness 0·216 mm. Spectra taken in a Perkin-Elmer-Spectrophotometer, Modell 21, with NaCl-prism; resolution 4, response 1/1, speed 2 min/μ, suppression 1.

emphasized the electrocortin-like properties of this agent. SINGER *et al.*, moreover, found an activity in the urine of patients with toxaemia of pregnancy, confirming thus the early findings of CHART *et al.*[1]. These authors later demonstrated the factor also in urine during cirrhosis of the liver with ascites[2].

After the isolation of the hormone from adrenals in crystalline form[3] and the elucidation of its structure[4], which suggested the name of aldosterone instead of electrocortin, the task was commenced by our groups of isolating the active substance from urine fractions with corresponding biological, physical and chemical properties. This work has now yielded pure aldosterone in crystalline form[5], proving that the sodium-retaining substance in urine is identical with the most potent known adrenocortical hormone.

The starting material was a 13 days' collection of urine (14·5 l, with a very low content of sodium) of a 10-year-old male nephrotic with massive oedema, who had not previously been treated with steroids. This was extracted (J.A.L.)[6] after acidification with hydrochloric acid to pH 1·0 within 40 min with a rather small quantity (4 times 0·15 Vol) of chloroform. The extract A, obtained in this way, upon washing with cold 0·1 n-sodium hydroxyde solution and water, drying (Na_2SO_4) and evaporating in vacuo at 40°C yielded a biologically active product. In paper chromatograms with ZAFFARONI's[1] and BUSH's[2] systems it showed a characteristic spot, which, according to its mobility, UV-absorption, reduction of blue tetrazolium, and formation of mono- and diacetates contained aldosterone. This fraction moreover was oxidized with periodic acid and yielded crude lactone 874[3]. All these substances were detected by paper chromatography.

To isolate aldosterone itself, the acid urine after 24 hours' standing at 20°C was extracted again in a similar way with chloroform. This extract *B* contained much more biologically active material[4] and was paper-chromatographed in the ZAFFARONI system[1] with propylene glycol-toluol. The band with slightly faster running properties than cortisone was eluted with 95% ethanol, and this fraction again chromatographed on paper in BUSH's *B*2-system (toluene-light petroleum-methanol-water[2]). The biologically active band yielded, with 95% ethanol, an amorphous eluate (4·6 mg), containing, according to its UV-absorption, about 2–3 mg of α,β-unsaturated ketones.

For further purification (R.N. and A.W.) this material was dissolved in acetone and yielded 4·4 mg of solubles. Twice 100 γ of these were run for analysis in the BUSH C-system (toluene-ethyl acetate-methanol-water)[2] at 40°C. The main spot, consisting of about 40% of the extract and being characterised with UV-absorption,

[1] J. J. CHART, E. G. SHIPLEY, and E. S. GORDON, Proc. Soc. Exper. Biol. Med. *78*, 244 (1951). – E. S. GORDON, J. J. CHART, D. HAGEDORN, and E. G. SHIPLEY, Obstetr. and Gynecol. *4*, 39 (1954). – Compare also C. W. LLOYD *et al.*, J. Clin. Invest. *31*, 1056 (1952).

[2] J. J. CHART and E. G. SHIPLEY, J. Clin. Invest. *32*, 560 (1953); see also A. M. BONGIOVANNI and W. J. EISENMENGER, J. Clin. Endocrinol. *11*, 152 (1951).

[3] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, and T. REICHSTEIN, Exper. *9*, 333 (1953); with O. SCHINDLER, Helv. chim. Acta *37*, 1163 (1954).

[4] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, Exper. *10*, 132 (1954); Helv. chim. Acta *37*, 1200 (1954).

[5] Result preliminarily cited by A. WETTSTEIN and G. ANNER, Exper. *10*, 401 (1954).

[6] The valuable collaboration of B. J. AXELRAD, A. DOWDY, J. HARVEY, and W. LEW is gratefully acknowledged.

[1] A. ZAFFARONI, R. B. BURTON and E. H. KEUTMANN, Science *111*, 6 (1950). – R. B. BURTON, A. ZAFFARONI, and E. H. KEUTMANN, J. Biol. Chem. *188*, 763 (1951).

[2] I. E. BUSH, Biochem. J. *50*, 370 (1952).

[3] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, Exper. *10*, 132 (1954); Helv. chim. Acta *37*, 1200 (1954).

[4] The valuable collaboration of B. J. AXELRAD, A. DOWDY, J. HARVEY, and W. LEW is gratefully acknowledged.

blue tetrazolium, sodium hydroxide, phosphoric acid[1] and antimony trichloride, showed the same R_F value as aldosterone (and 17-hydroxy-corticosterone) and contained only minor impurities. This system was therefore used to purify 4·2 mg of the solubles preparatively on paper, the band corresponding to aldosterone being eluted with 20% methanol. The eluate was evaporated *in vacuo* and extracted 8 times with chloroform, yielding a residue of 3·4 mg. Upon solution in a little moist ether-acetone (3:1) and inoculation with aldosterone it crystallised at once. After 15 hours' standing at −10°C we decanted the mother liquor and washed the crystals 3 times with ether-acetone (3:1). The preparation (1·0 mg) melted at 100–110°C, crystallised again partially upon further heating and melted finally at 150–155°C. Recrystallisation was carried out with a big loss from ether-acetone (2:1) with the purpose of obtaining the purest possible product. It yielded 0·35 mg of pure aldosterone which melted partially at 110–115°C, crystallised again totally upon further heating and melted finally at 156–165°C. The melting point of a mixture with authentic aldosterone showed no depression. The infrared spectrum in chloroform[2] was in conformity with that of aldosterone from beef adrenals[3] (compare Fig. 1, *a* and *b*). The broad band at 2·85–3·05 μ, shown only in the latter, is due to the extensive hydrogen bonding, occurring only in high concentration[3], this being in the case of the aldosterone from adrenals about 5 times higher than in the case of the aldosterone from urine.

The mother liquor of the first crystallisate contained, according to paperchromatographic determination, a further 0·9 mg of aldosterone. Extract *B*, therefore, contained a total of 1·9 ± 0·2 mg aldosterone and the entire 13 days' urine probably towards 3 mg of aldosterone. The high content, together with the comparatively simple isolation process in this case, makes the urine of nephrotic patients a very suitable starting material for the extractive preparation of aldosterone. The physiological significance of the high aldosterone excretion in certain diseases has still to be established.

A detailed report will appear elsewhere.

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Zusammenfassung

Es ist erstmals gelungen, Aldosteron aus menschlichem Urin zu isolieren. Eine Fraktion des 13-Tage-Harnes von einem Knaben mit Nephrose und massiven Ödemen ergab 1,0 mg dieses biologisch hochaktiven Hormons in kristallisierter Form. Da der Gesamtgehalt gegen 3 mg betrug, stellt solcher Harn ein geeignetes Ausgangsmaterial dar zur extraktiven Gewinnung von Aldosteron. Letzteres erwies sich in seinen Eigenschaften als identisch mit Aldosteron aus Rinder-Nebennieren.

[1] R. NEHER and A. WETTSTEIN, Helv. chim. Acta *34*, 2278 (1951).

[2] Our best thanks are due to Dr. E. GANZ, Basle, for these determinations.

[3] S. A. SIMPSON, J. F. TAIT, A. WETTSTEIN, R. NEHER, J. v. EUW, O. SCHINDLER, and T. REICHSTEIN, Helv. chim. Acta *37*, 1173–1176 (1954).

Neue Synthesen des Chloramphenicols und deren stereochemischen Beziehungen

Wir haben zwei neue Synthesen des Chloramphenicols (I) ausgearbeitet. Diese Synthesen geben auch bestimmte Aufklärung über stereochemische Zusammenhänge in der Reihe der β-Phenylserinäther und über den «Neighboring group effect»[1].

Vom Diastereoisomeren des β-Phenylserin-O-methyläthers (II) mit dem niedrigeren Schmelzpunkte ausgehend (Darstellung durch Ammonolyse von 2-Phenyl-2-methoxy-1-brompropionsäure Smp. 140°[2]), wurde die threo-Modifikation des β-Phenylserinolmethyläthers (III), (Smp. des N-p-Nitrobenzoats: 179-181°, $C_{17}H_{18}O_5N_2$: berechnet C 61,85, H 5,49, N 8,48; gefunden C 61,95, H 5,63, N 8,50) auf zwei Wegen hergestellt: erstens wurde der Äthylester von (II) mit $LiAlH_4$ reduziert, zweitens wurde das Säurechlorid der Phthalylverbindung von (II) nach ROSENMUND zum 2-Phenyl-2-methoxy-1-phthalimino-propionaldehyd reduziert, dann mit Aluminiumisopropylat reduziert und mit Hydrazin dephthalyliert. Das O-N-Diazetat von (III) wurde nitriert und desazetyliert; die entstandene Base (IIIa, Smp.: 82–84°, $C_{10}H_{14}O_4N_2$: ber. C 53,09, H 6,20; gefunden C 53,04, H 6,22) gab nach Demethylierung das threo-1(p-Nitrophenyl)2-amino-1,3-dioxypropan. (IIIa) wurde mit Weinsäure oder Dibenzoylweinsäure in die optischen Antipoden gespalten. Das linksdrehende Isomere von (IIIa) (Smp.: 105–107°, $[\alpha]_D$–74°, C = 1% in n-Salzsäure) konnte zu einer Verbindung demethyliert werden, die mit der Base – entstehend bei der Hydrolyse des natürlichen Chloramphenicols (Ia) – in jeder Hinsicht identisch war. (Ia) gab mit Pentachlorazeton in ausgezeichneter Ausbeute Chloramphenicol.

Das Diastereoisomere des β-Phenylserin-methyläthers (IV) (mit dem höheren Schmelzpunkt; darstellbar durch Ammonolyse der 2-Phenyl-2-methoxy-1-brompropionsäure vom Smp.: 180° (III), welche sich in quantitativer Ausbeute aus trans-Zimtsäure in methylalkoholischer Lösung durch die Einwirkung von Brom und gelbem Bleioxyd bildet[3]), konnte mit denselben Methoden reduziert werden. Es entstand so der erythro-β-Phenylserinol-methyläther (V) (Smp. des N-p-Nitrobenzoats 163–164°; $C_{17}H_{18}O_5N_2$: berechnet N 8,48; gefunden N 8,26), welcher wie oben in die p-Nitroverbindung übergeführt werden konnte (Va) (Smp.: 110–111°, $C_{10}H_{14}O_4N_2$: berechnet C 53,09, H 6,20, N 12,40; gefunden C 52,87, H 6,22, N 12,36). Interessanterweise bildete sich beim Demethylieren von (V) mit wässriger Bromwasserstoffsäure neben erythro-β-Phenylserinol in erheblicher Menge auch threo-β-Phenylserinol. Um diese Erscheinung zu erklären, unterwarfen wir erythro-β-Phenylserinol den Demethylierungsbedingungen (Kochen mit wässriger Bromwasserstoffsäure); wir bekamen so in guter Ausbeute das threo-Isomere (das erythro- oder threo-β-p-Nitrophenylserinol und das threo-β-

[1] S. WINSTEIN und R. E. BUCKLES, J. Amer. Chem. Soc. *64*, 2780 (1942). – E. ABDERHALDEN und W. ZEISSET, Z. Physiol. Chem. *200*, 179 (1931).

[2] H. E. CARTER und E. VAN LOON, J. Amer. Chem. Soc. *59*, 2555 (1937); *60*, 1079 (1938).

[3] Die oben erwähnte neue Methode wurde mit gutem Erfolg zur Addition der Elemente der Alkylhypobromite bei anderen ungesättigten Verbindungen gebraucht. So zum Beispiel bei ungesättigten Alkoholen (Zimtalkohol), bei ungesättigten Aldehyden und Ketonen (Zimtaldehyd, Benzalazeton), bei ungesättigten Säuren (2,2-Dimethylacrylsäure); die Ausbeuten erreichten in jedem Falle 90%. Die Methode ist auch brauchbar zur Herstellung von Äthoxy-Brom-Verbindungen, aber hier kann man gute Ausbeuten nur durch Zugabe katalytischer Mengen Wassers erreichen.