passage of oxygen and carbon dioxide, the role of which may be critical.

Conclusion. The model of the epidermis sketched in this paper, approximate as it is, exhibits a sufficient degree of internal consistency to account for both the maintenance and repair of the normal epidermis, without necessarily negating the modulating influence of cellular products of injury. The model is based on the supposition that the behavior of the population of epidermal cells can be described in a simple kinetic manner and that its growth depends on the amount of nutrients it receives, both by diffusion and bulk transport. The numerical examples presented in the paper are based on data which are firmly established only in some cases, consequently the interpretation of the results must be correspondingly fluid. In spite of this, the general conclusions may be valid and the model may furnish a clue to the psorigenic process, which it is felt, illustrates par excellence the consequences of a chronic impairment of the capacity to rapidly form an adequate skin barrier to water[29,30].

Résumé. Un modèle cinétique simple a été développé pour l'épiderme; les lois cinétiques de premier ordre peuvent lui être appliquées fructueusement. Il est envisagé que la régulation homéostatique de l'épiderme peut se faire par l'ajustement des quantités de nourriture qui lui parviennent. Le flux nutritif atteignant l'épiderme est composé du flux de diffusion, qui prédomine normalement, et du flux actionné par l'évaporation, à la surface de la peau, de l'eau provenant de l'ultra-filtrat du sérum. Ce dernier pourrait prédominer quand l'épiderme aurait été lésé, et serait augmenté quand la peau ne constituerait pas une barrière suffisante au passage de l'eau.

Par analogie avec les cultures de microbes en «steady state», il est envisagé que le nombre total de cellules épidermiques par unité de surface cutanée et leur taux de croissance sont des fonctions du taux du transfert de nourriture à l'épiderme. L'équation hyperbolique de MONOD est donc modifiée et appliquée à l'épiderme. Une évaluation approximative de cette équation suggère que le flux nutritif vers l'épiderme est augmenté en cas de psoriasis aussi bien que dans la peau normale lorsqu'elle est dépouillée du stratum corneum. On considère que dans le psoriasis le dérangement cinétique de l'épiderme et le dérangement fonctionnel de la barrière de la peau au passage de l'eau sont intimement liés.

E. ASCHHEIM

Department of Dermatology, Stanford University School of Medicine, Palo Alto (California 94394, USA), 19 May 1967.

[29] This research was supported by N.I.H. Research Grant No. HE 03833-08. The author is a recipient of N.I.H. Research Career Development Award No. HE 12, 476-03.

[30] I would like to thank Prof. H. O. FUCHS of the Department of Mechanical Engineering of Stanford University for suggesting that equation (3) represents a valid approximation.

The Identification of Chlorpromazine Metabolites in Human Blood by Gas Liquid Chromatography

Chlorpromazine (CP) has been used since 1952 in the treatment of mental illness[1]. It has been estimated that more than 50 million people have taken the drug. Although several metabolites have been identified in urine, practically nothing is known about the metabolites in blood.

Previous attempts have been made to estimate chlorpromazine and its metabolites both as conjugates and non-conjugates. The spectrophotometric method used in these studies must be regarded as unsatisfactory due to its lack of specificity and sensitivity, and especially due to its inability to determine single metabolites[2].

In this communication we describe the use of gas liquid chromatography (GLC) for the analysis of chlorpromazine and its metabolites in human plasma and red blood cells.

Experimental. Reference compounds and reagents: chlorpromazine, desmethylchlorpromazine (DMCP), didesmethylchlorpromazine (DDMCP), and 3-(2-chloro-10-phenothiazinyl)propionic acid (Cp-prop), were kindly supplied either by Leo Pharmaceutical Company, or the NIMH. β-glucuronidase (containing sulfatase), Sigma. Trifluoroacetic anhydride (TFAA), Fluka. Pentafluoropropionic anhydride and Heptafluorobuturic anhydride, K & K Laboratories, Inc. Chloroacetic anhydride and Dichloroacetic anhydride, Eastman Organic Chemicals. Dichloromethane, Dimethylformamide, and Toluene Allied Chemical. Heptane, Hopkin & Williams Ltd. Isoamylalcohol, E. Merck AG.

All analyses were carried out on an Aerograph 204 gas chromatograph, equipped with electron capture detectors operated at 90 V. The whole system was in glass. Nitrogen was used as carrier gas. Inlet pressure was kept at 5.5 kg/cm² and flow rate at about 30 ml/min. The length of the $^1/_8$ inch coiled glass columns was about 1.5 m.

Gas Chrom P, Chromosorb G (both acid washed and treated with 5% dimethyldichlorosilane) or Gas Chrom Q were used as supports.

Gas Chrom P and Q were used in combination with 3% Versamid 900 or Versilube F-50 and Chromosorb G with the same liquid phases in the concentrations 0.75–1.5%.

Extraction methods. Blood samples (15–30 ml) were taken from patients who received 100–250 mg CP/day. After centrifugation, the plasma sample was extracted twice at pH 12 with an equal volume of dichloromethane. This extract contains the basic non-conjugated metabolites. The same plasma was then adjusted to pH 3.8 and incubated overnight at 37 °C and with gentle shaking with β-glucuronidase containing sulfatase. The pH of the plasma was then adjusted to 12 and it was again extracted twice with dichloromethane. This extract contains the hydrolyzed conjugates with basic character.

The pH of the plasma was then adjusted to 1.5 and i was extracted twice with an equal volume of dichloromethane. This extract contains the acidic metabolites (non-conjugates and/or hydrolyzed conjugates). No acid extraction was attempted before the incubation, because proteins of the plasma become partly precipitated at such low pH-values.

[1] J. DELAY, P. DENIKER and J. M. HARI, Annls méd.-psychol. *2*, 112 (1952).

[2] C. L. HUANG and B. H. RUSKIN, J. nerv. ment. Dis. *139*, 381 (1964).

In some cases the erythrocytes were also investigated. They were washed twice with about 100 ml of 0.9% sodium chloride and hemolysed with water of the same volume as the plasma. The pH was then adjusted to 12 and the red blood cells extracted twice with dichloromethane. Incubation with β-glucuronidase at pH 3.8 was followed by both a basic and acid extraction. For chlorpromazine itself we have also used a previously described extraction procedure[3], which starts with an extraction into heptane containing 1.5% isoamylalcohol. This is followed by washing with acetate buffer at pH 5.6 and extraction into 0.1 *N* HCl. The acid water phase is made alkaline and extracted with toluene containing 15% isoamylalcohol.

Derivative formation. Trifluoroacetic anhydride was used exclusively for making derivatives of the basic extracts with dichloromethane as solvent and dimethylformamide (3%) as catalyst. All samples were left overnight at room temperature and then evaporated to dryness under a stream of nitrogen. The extract was redissolved in 10–20 µl of methanol. Methyl-esters of the acidic compounds were prepared with diazomethane in ether with methanol as a catalyst.

Other derivatives, which will be treated in the discussion, were prepared using the same solvent and catalyst as was used for the trifluoroacetamides.

Results. Non-conjugated metabolites: gas chromatographic analysis of a TFAA-treated dichloromethane extract of plasma showed peaks with the same retention times as those of synthetic *N*-trifluoroacetyl-*N*-desmethylchlorpromazine (DMCP-TFA), and *N*-trifluoroacetyl-*N*-didesmethylchlorpromazine (DDMCP-TFA). The heptane-toluene extract of plasma gave a peak with the same retention time as chlorpromazine (Figure 1). Extracts of several plasma samples from control individuals, who were not on drug therapy, did not show any peaks with retention times identical to the ones above.

Conjugated or bound metabolites: the extract of hydrolyzed plasma was also treated with TFAA and analysed by GLC (Figure 2). Greater amounts of the desmethylated compounds were found in this fraction than in the non-conjugated fraction. Some of the acid extracts after treatment with diazomethane showed a peak with the same retention time as that of synthetic methyl-3-(2-chloro-10-phenothiazinyl)propionate (Cp-prop-Me-ester). The retention time of the ester relative to chlorpromazine is 1.6. No normal plasma extract showed any peak with the same retention time as the above-mentioned.

Red blood cells: the erythrocytes from some of the blood samples were also investigated. No non-conjugated metabolites were found. After hydrolysis with β-glucuronidase 2 peaks with the same retention times as DMCP-TFA and DDMCP-TFA were found.

Discussion. Previous investigations of chlorpromazine in body fluid using GLC have been limited to use of the flame ionization detector (FID)[4,5], or the microcoulorimetric detector[6,7]. The former is a good allround detector for biological extracts[8]. The microcoulorimetric detector

[3] N. P. Salzman and B. B. Brodie, J. Pharmac. exp. Ther. *46*, 108 (1956).
[4] J. L. Driscoll, H. F. Martin and B. J. Gudzinowicz, J. Gas Chromatography *2*, 109 (1964).
[5] R. M. Rose, A. DiMascio and G. L. Klerman, J. psychiat. Res. *2*, 299 (1964).
[6] D. E. Johnson, C. F. Rodriguez and H. P. Burchfield, Biochem. Pharmac. *14*, 1456 (1965).
[7] C. F. Rodriguez and D. E. Johnson, Life Sci. *5*, 1283 (1966).
[8] *Biochemical Application of Gas Chromatography* (Ed. H. A. Szymanski, Plenum Press, N.Y. 1964).

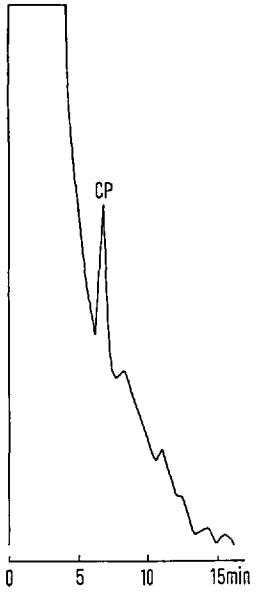

Fig. 1. Gas chromatogram of a heptane-toluene extract from plasma drawn 1 h after administration of 100 mg chlorpromazine. GLC-conditions: 3% Versamid 900 on Gas Chrom P (100–120 mesh). Injector temp. 230 °C. Column temp. 220 °C. Detector temp. 225 °C.

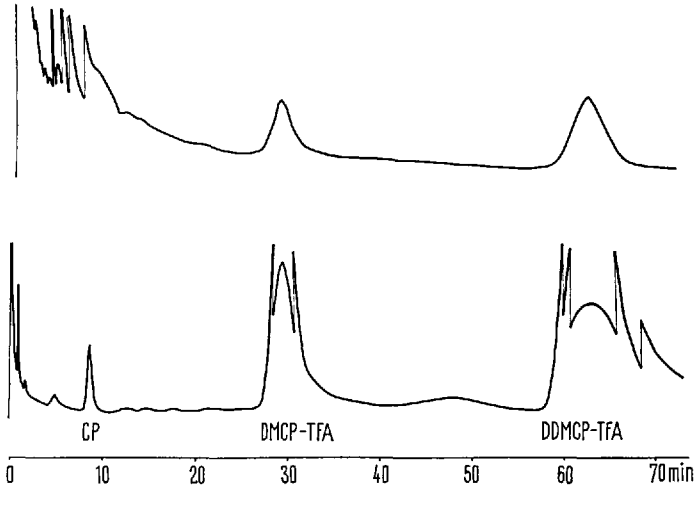

Fig. 2. Gas chromatographic separation of chlorpromazine metabolites in human plasma. GLC-conditions: Same as in Figure 1. Upper panel: Gas chromatogram of a basic TFAA-treated extract of hydrolysed plasma from a patient receiving 150 mg chlorpromazine per day. Lower panel: Reference compounds: Chlorpromazine (CP), *N*-Trifluoracetyl-*N*-desmethylchlorpromazine (DMCP-TFA) and *N*-Trifluoracetyl-*N*-didesmethylchlorpromazine (DDMCP-TFA) chromatographed under identical conditions.

estimates quantitatively one element of an organic compound e.g. chlorine. The FID, under our conditions, is sensitive in the range of 10^{-7}–10^{-8} g. The maximal practical sensitivity of the microcoulorimeter for chlorpromazine metabolites is about 10^{-7} g of each compound[6].

The electron capture detector (ECD) has been successfully used for the determination of insecticides containing organically bound chlorine[9]. It would thus be reasonable to assume that it could be of use also for the analysis in the body of chlorpromazine and its metabolites. It has already been demonstrated that chlorpromazine can be detected in ng quantities by the ECD[6].

Under our experimental conditions CP could be detected in quantities of 10^{-9} g and the desmethylated metabolites as TFA-derivatives in the range of 10^{-11} g. The ECD is more specific and more sensitive than the FID. Its disadvantages are: a maximum temperature of 225 °C, risk of contamination of the detectors, and the fact that only a small part of the response curve is linear. In contrast to other reports[10], we could not substantiate that changing of the ECD-voltage had any effect upon the sensitivity.

The successful GLC determination of CP and its metabolites is primarily dependent upon 3 factors, the extraction procedure, the choice of suitable derivatives, and the choice of gas chromatographic conditions including support, liquid phase, temperature etc.

Dichloromethane has been used with advantage for the extraction of chlorpromazine and its metabolites from urine[6]. We have also found it to be a suitable extraction medium for CP and its non-polar metabolites in plasma. Dichloromethane, however, is not a very discriminating solvent and the gas chromatograms invariably have large fronts, disguising CP which has a relatively short retention time (Figure 2).

The previously mentioned extraction procedure with heptane-toluene[3] used directly with the plasma or with the dichloromethane extract allows detection of CP as well as its non-polar metabolites. If one is primarily interested in the desmethylated metabolites, the dichloromethane extraction procedure is preferable because it is less time-consuming. The metabolites are completely resolved under the GLC-conditions used. When the metabolites after incubation are analysed CP has already been removed by the first extraction procedure for the non-conjugated compounds. CP itself is the only compound in the series possible to determine gas chromatographically without derivative formation under the conditions described above.

Previous gas chromatographic studies have used the acetyl derivatives of the desmethylated compounds[4–6]. For many reasons, however, the trifluoroacetyl derivatives seem to be more suitable. Trifluoroacetic anhydride is more reactive than acetic anhydride. In addition the trifluoroacetamides have shorter retention times than the corresponding acetamides and also show less tendency to tail. Trifluoroacetylation increases the sensitivity of the ECD to the CP-derivatives about 100 times. Introduction of the TFA-group affects the electron density of the molecule so that it has a greater capacity for capturing electrons. The TFA-group itself is said to have very little effect upon the electron capturing activity. The use of pentafluoropropionyl derivatives does not increase the sensitivity. With heptafluorobuturyl derivatives the sensitivity is increased about 5–10 times as compared to the the TFA-derivatives. However, with these derivatives there is greater difficulty in evaporation and concentration of the solutions.

Mono- and dichloroacetic anhydride have also been tested as acylating agents but are less satisfactory because of prolonged retention times. The monochloroacetyl derivatives gave no increase in sensitivity as compared to the TFA-derivatives, while the dichloroacetyl derivatives gave a remarkable increase in the sensitivity.

So-called polar stationary phases were found suitable for gas chromatographic separation of these chlorophenothiazines. Versamid 900 was found to be the best overall phase, in spite of the fact that some metabolites have a long retention time.

Chlorpromazine could be demonstrated in ng quantities in plasma taken from patients 1 h after the administration of 50 mg of the drug. The parent compound and the desmethylated non-conjugates seem to disappear from the blood within a few hours. By contrast the bound desmethylated products could be demonstrated in the plasma at least 6 h after administration of CP. The ratio between the 2 desmethylated compounds was different from one patient to another. Certain amounts of the bound desmethylated products were also found in the red blood cells. Chlorophenothiazinyl-propionic acid could be identified in the plasma of some patients but was completely absent in others. The nature of the bound metabolites is unknown at the moment, but work is in progress to determine if they are *N*-glucuronides, salts of glucuronic acid, or merely bound to protein.

The demonstration of the various metabolites raises the important question whether chlorpromazine acts by itself or through one or several of its metabolites. The parallel with imipramine and its active metabolite desmethylimipramine is obvious[11]. Attempts to correlate the excretion pattern of metabolites of chlorpromazine with the clinical response to the drug therapy have been made by GREEN et al.[12]. They report less of the conjugates in urine of people who respond poorly to the drug. Plasma levels are generally considered to be more reliable as a base line for the estimation of therapeutic effects and side effects of drugs than the occurrence of metabolites in urine. The analyticyl method presented above will facilitate clinical pharmacological studies with CP and related compounds[13].

Zusammenfassung. Chlorpromazin und 5 seiner Metaboliten wurden gaschromatographisch in menschlichem Plasma identifiziert. Zwei dieser Metaboliten wurden auch in Erythrozyten aufgefunden. Die Blutproben wurden von Patienten genommen, welche täglich 100–250 mg Chlorpromazin erhielten. Chlorpromazin und die nicht konjugierten Metabolite scheinen vom Plasma innerhalb weniger Stunden nach Verabreichung einer Dosis Chlorpromazin zu verschwinden. Die gebundenen Metabolite konnten jedoch zu jeder Zeit nachgewiesen werden. Das metabolische Bild kann von einem Patienten zum anderen wechseln.

C.-G. HAMMAR and B. HOLMSTEDT

Department of Toxicology, Swedish Medical Research Council, Karolinska Institutet, Stockholm 60 (Sweden), 26 July 1967.

[9] E. J. BONELLI and K. P. DIMICK, in *Lectures on Gas Chromatography–1964* (Eds L. R. MATTICK and H. A. SZYMANSKI; Plenum Press, N.Y. 1965), p. 15.

[10] B. J. GUDZINOWICZ, J. Gas Chromatography *4*, 110 (1966).

[11] W. HAMMER and F. SJÖQVIST, Life Sci. *6*, 1895 (1967).

[12] D. E. GREEN, I. S. FORREST, F. M. FORREST and M. T. SERRA, Expl Med. Surg. *23*, 278 (1965).

[13] Acknowledgment. This work has been supported by a grant from the National Institute of General Medical Sciences (No. GM 13978) to Prof. B. HOLMSTEDT and Assistant Prof. F. SJÖQVIST, Karolinska Institutet.