Résumé. La glande tyroide du *Msytus vittatus* sous photoperiodes normales montre des variations cycliques saisonnières: Au cours d'un an il y eut 2 phases d'activité tyroidale augmentée et chacune fut séparée par 1 phase de repos. Sous illumination prolongée le type de variations resta identique; sauf les phases de repos furent enregistrées 2 mois plus tôt que la normale. Ce plan rythmique d'activités disparut dans une obscurité totale et l'activité tyroidale demeura a un niveau élevé toute l'année.

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STUDIORUM PROGRESSUS

Epidermal Homeostasis - a Numerical Model. Kinetics of Epidermal Cells

With the possible exception of the nervous tissue, the maintenance of functional capacity of adult organs involves a continuous process of cell renewal. In the majority of cases this process depends on the persistence of low level mitotic activity, but it may involve cellular transformation as when antigenic stimulation induces plasma cell formation. An important aspect of the problem is that, in general, mitotic activity keeps pace with cell destruction in such a manner that there tends to be maintained a rather constant ratio between the mass of a given organ and the total body mass. This regulation appears to be the rule, although in some instances physiological cyclical activity or altered functional demand obscures this basic relation, as in the case of the uterus during menses or pregnancy.

The manner in which mitotic activity keeps pace with cellular destruction is not understood and may involve a variety of mechanisms. In the case of the liver or kidneys, regeneration experiments suggest that functional load is the determining factor. A similar conclusion may apply to the regulation of the mass of red cells, since either bleeding or injection of additional red cells is compensated for, the mass of red cells returning to the pre-experimental level[1].

A kinetic model of growth regulation developed by WEISS and KAVANAU interprets cellular homeostasis in terms of production of inhibitory or repressive substances which are able to diffuse across cell boundaries[2]. BULLOUGH has likewise postulated an inhibitor-based negative feedback to account for the maintenance of the normal epidermis[3], and has subsequently claimed to have isolated the repressor substance. IVERSEN incorporated these ideas in an electronic analogue and demonstrated that an inhibitor-based negative feedback system does indeed reproduce the kinetic behavior of epidermal cells under a variety of conditions[4].

Suggestive as these ideas are, there is no satisfactory evidence to substantiate them. IVERSEN's analogue simulation does not per se prove the existence of a molecular repressor mechanism, since the postulated model is descriptive of a number of different physical systems. BULLOUGH's evidence is rather unsatisfactory, mainly because the postulated repressor does not repress unless epinephrine is present in the system. The lowest dose of epinephrine employed in vivo[5] is so large (10 μg/mouse) as to exceed thousands of times the normal blood content of this amine, (less than 1 μg/l) suggesting that mitotic inhibition may have resulted from the combined effects of adrenocortical activation and intense prolonged cutaneous vasoconstriction. The in vitro experiments are similarly inconclusive because they were conducted in an atmosphere of pure oxygen[5,6] which favors the oxidation of epinephrine to adrenochrome, a known mitotic poison. This consideration is more than suggestive, since addition of reduced glutathione or ascorbic acid which by themselves do not affect mitosis, effectively prevents or abolishes the presumed antimitotic effects of epinephrine[7].

This short appraisal of current views highlights our surprisingly unsatisfactory grasp of factors involved in epidermal homeostasis. An attempt to re-evaluate the problem de novo has resulted in a basically simple kinetic formulation.

Epidermal nutrition. In contrast to other tissues, the epidermis does not possess a blood supply of its own, depending for its non-gaseous needs on the movement of substances across the basement membrane. Thus the nutritive flux into the epidermis could be governed both by the extent of exchange of materials between blood and tissue fluid in the dermis and by the magnitude of the subsequent transport of metabolites across the dermal-epidermal junction.

Since transendothelial exchange is flow limited both in the case of small[8] and large[9] molecules, it is subject to hemodynamic regulation. However, since at low blood flows local metabolic regulation may take precedence over central constrictor influences[10], it is unlikely that under normal conditions insufficient transendothelial exchange can last long enough to materially affect the nutritive traffic across the basement membrane.

Thus, assuming an adequate transendothelial movement of metabolites in the dermis, epidermal nutrition becomes a function of the magnitude of metabolite flux across the basement membrane. This process is usually thought to be due to diffusion, but under certain conditions it is possible to distinguish the presence of another component of transport, which is mediated by the bulk flow of water. This component may readily be recognized in scotch tape stripped skin, where the surface becomes

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covered with a layer of serum ultrafiltrate from which water evaporates readily. The epidermis in this situation acts like a wick, evaporation causing a constant flow of solutes onto the surface where they tend to accumulate. This process differs from diffusion, since it can occur in the absence of any solute concentration gradients, being obviously coupled to the activity gradient of the solvent at the vapor-liquid interface. Such wick action would result in the establishment of an activity gradient of the solute, and its diffusion in the opposite direction, with a zero net solute transport once a steady state is established. In the presence of a metabolic or other sink for the solute, as in the epidermis, diffusion into the epidermis and bulk flow-mediated transport may both occur in the same sense.

The dual character of transport into the epidermis should lend itself to description in terms of ONSAGER's theory[11], since the total flux of a given metabolite can be resolved into at least 2 component fluxes.

The relative contribution of these 2 modes of nutrient transport is difficult to establish. The bulk flow-mediated component is probably minimal under normal conditions, but very likely becomes prominent in situations in which the skin barrier to water is functionally deficient, or in general, when the evaporative loss from the skin surface is large. The resulting total nutrient flux may then exceed that of normal epidermis and lead to cellular proliferation.

Epidermis as a steady state system. The maintenance of a relative numerical constancy among the various cell types of the epidermis in the face of uninterrupted cell loss and cell division suggests that the population of epidermal cells represents an open steady state system. The time invariant steady state concentration of the various cellular components of such a system would then be determined by the magnitudes of the various rate constants and not by the initial composition, a property which should endow the system with so-called equifinality, i.e. the ability to recover following a perturbation[12]. Such a perturbation may consist of a dimunition in the size of one or more cellular compartments as in injury or of an increase, a situation which does not appear to have been experimentally studied in the epidermis, and the restoration may or may not be accompanied by an overshoot. Accordingly, any transient or permanent change in the size or composition of the population of epidermal cells must be due to an appropriate change in one or more of the relevant rate constants of the system.

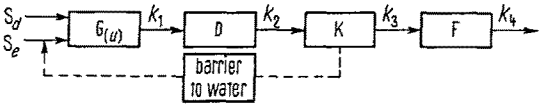
Among the various rate constants, the rate of division of basal cells has received particular attention, since epidermal homeostasis could be effected by means of mitosis promoting[13,14] or mitosis repressing[2,3] agents. However, the mitotic rate of epidermal cells could be influenced by other factors as well, such as temperature or rate of supply of a limiting metabolite. The effects of environmental temperature on cell mitosis in the skin appears well established[15,16], there is no evidence however, for a Malthusian type of control of epidermal mitotic activity except perhaps in the limiting case of ischemic disorders in which the skin exhibits various degrees of atrophy. However, nutritional control is prominently involved in the continuous cultivation of micro-organisms in which a constant cell density is maintained. In such cultures various steady state population densities can be established at will, the regulation being based on the dependence of the specific growth rate of the organism on the rate of supply of a limiting metabolite[17,18]. The experimentally-established occurrence of this control mechanism in one system strongly favors, in our opinion, its consideration in epidermal homeostasis in preference to other, less founded hypotheses.

To recapitulate, it is believed that the epidermis can best be conceived as an open steady state system through which flow both matter and energy and that this feature accounts satisfactorily for its self-regulating properties. In the overall process of conversion of a given input of metabolites into keratin a change in the steady state level of any given cellular constituent of the epidermis may occur only in virtue of a change in one or more of the relevant rate constants. It is further considered that the biological control of the epidermis is based on the circumstance that both the total number of epidermal cells and the rate of production of new cells per unit area of skin surface depend on the rate at which the epidermis is supplied with a limiting metabolite, and that the numerical expression of this relationship is approximated by the hyperbolic function found by MONOD to apply in the case of continuous cultures of micro-organisms.

The formal presentation of these ideas is subdivided into 2 parts. In the first part, it will be shown that it is possible to describe epidermal growth in terms of first-order kinetics and to this end the differences in cellular outputs per unit area of skin surface of a number of epidermal populations of various sizes are expressed as differences in specific growth rates of an idealized epidermal cell population of standard size.

The adjusted specific growth rates are calculated both for psoriatic and scotch tape stripped skin, and the values are used in the second part of the paper to estimate by how much the nutrient flux into the epidermis must have increased above normal in order to account for the high rate of cell production which characterizes both of these situations.

The model. As a first approximation the epidermis can be considered as consisting of 4 conceptually discrete cellular compartments (Figure). The compartments are G (generating cells), D (differentiating cells), K (keratinocytes) and F (flakes). For simplicity the capital letters also designate the size (No. of cells) of the respective compartment per unit area of skin surface. The number of compartments of this model has no special significance; some of these could be lumped together, or further subdivided if one so wished. Also, no special significance should be attached to the names assigned to the compartments; they only serve to designate successive stages in the progression from basal cells to the scaly agglomerates which are shed from the skin surface.



First approximation to the kinetic description of the epidermis. Compartment F is shown distinct from compartment K in order to emphasize that the rate of scaling k_4F may be abnormal. The meaning of symbols is given in the text.

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The transfer of cells among the various compartments is essentially unidirectional, the respective rate constants being k_1, k_2, k_3 and k_4, the latter representing the rate of desquamation; u is the specific growth rate of G, given by

$$u = (1/G)\ (dG/dt) \tag{1}$$

The arrows on the left side represent the rates of supply of the limiting substrate by diffusion (S_d) and by evaporation-activated bulk flow (S_e). In this model the specific growth rate u is taken to depend solely on the sum of S_d and S_e. The negative feedback is represented by the broken arrow, the sign reverter being the water barrier function of K. This function may be represented as a fraction of K when comparison is made between skins obtained from various locations, but for the same location it is likely to be determined by the degree of compaction of cells of compartment K.

In general, the condition of the skin is inferred from observable events at the surface of the epidermis. In particular, the rate of shedding of scales from a unit area of skin surface mirrors the extent of the mitotic activity of the generative cells which normally line the basement membrane. Since the area of the basement membrane is in many cases larger than the area of the overlying surface of the skin and since in some situations, as in psoriasis, the generative cells are found in more than one cell layer[19], the size of population G per unit area of skin surface is not constant. For this reason we consider an equivalent population P of standard size and specific growth rate z which may be larger than u. The cells of this population are considered to form a single layer which lines an area of the basement membrane equal to the surface area of the overlying skin.

The original population G is related to the standard population P by the relation

$$P = G(A_s/A_j\ L) \tag{2}$$

where A_s and A_j represent the area of the surface of the skin and of the dermal-epidermal junction, respectively, and L is the number of layers occupied by the generative cells. As an approximation, the factor A_s/A_j is taken as equal to the ratio of the linear dimensions of the skin surface to the underlying basement membrane, as seen in histological preparations of cross sections of the skin, i.e.,

$$A_s/A_j = l_s/l_j \tag{3}$$

A necessary requirement is that the specific growth rates u and z be related in such a manner that during the time it takes the population P_0 to double its size, population G would also have produced a quantity of cells equal to P_0. This condition is satisfied when

$$z = u\ 0.693/\ln\left[(A_s/A_j\ L) + 1\right] \tag{4}$$

In the limiting case when the geometry factor $(A_s/A_j\ L) = 1$, $z = u$.

By transforming in this manner the actual population G into an equivalent population P we can express its behavior in terms of turnover, doubling and half times and use these parameters for comparative purposes.

The following equations may then be applied to the model:

$$dP/dt = z\ P - k_1\ P \tag{5}$$

$$dD/dt = k_1\ P - k_2\ D \tag{6}$$

$$dK/dt = k_2\ D - k_3\ K \tag{7}$$

$$dF/dt = k_3\ K - k_4\ F \tag{8}$$

The epidermis will be in a steady state when there is no change in the effective composition of the various compartments, i.e. when

$$dP/dt = dD/dt = dK/dt = dF/dt = 0 \tag{9}$$

which leads to

$$\underset{\text{rate of cell production in } G}{z\ P} = \underset{\text{rate of cell loss from } G}{k_1\ P} = \underset{\text{rate of production of keratinocytes}}{k_2\ D} = \underset{\text{rate of production of scales}}{k_3\ K} = \underset{\text{rate of desquamation}}{k_4\ F} \tag{10}$$

According to this equation the rate of scaling at the skin surface is equal to the rate of formation of new cells in compartment G.

The application of first-order kinetics to changes in compartment size is based on the assumption that the magnitude of a change in a given compartment is proportional to the size of the compartment, a not unlikely behavior of the system.

The integral form of equation (5) is

$$P_t = P_0 \exp{(z - k_1)\ t} \tag{11}$$

where P_0 and P_t represent the number of cells in compartment G at time zero and after the time interval t. Emplying the usual notation, the doubling time, half time and turnover time of this population are given by

$$t_d = 0.693/z \tag{12}$$

$$t_{1/2} = 0.693/k_1 \tag{13}$$

$$T = 1/z \tag{14}$$

Normal, stripped and psoriatic epidermis. The potential usefulness of the foregoing approach is shown as follows. If the specific growth rate of the normal epidermis is 0.0086/day[19] and the epidermis is of the 'flat' type (i.e. the geometry factor $A_s/A_j\ L = 1$), then from equation (4) $z = u$. Such epidermis should exhibit a turnover time $T = 116$ days and a doubling time $t_d = 80.6$ days (equations 14 and 12). Actual studies of osmium tetraoxide marked skin, approximate as they are, indicate that 25 epidermal cell layers on the flexor aspect of the forearm where the skin is stated to be of such 'flat' type, are renewed in 100 days[20].

However, work involving the incorporation of labeled materials into the epidermis of the human back indicates that the turnover time of this population of cells is 27 days[21]. If we accept this value, even though repeated scraping of the same skin sites may have accelerated cell renewal, then for this location, the values for normal skin are

$$z_{(\text{normal skin})} = 0.037\ (\text{days})^{-1} \tag{15}$$

and the doubling time $t_d = 19$ days.

With respect to abnormal situations, use can be made of a number of available data. Thus the removal of cornified cells from the flexor aspect of the forearm by multiple application of scotch tape (stripping) is reported to increase the mitotic activity of the epidermis per unit area of skin surface by a factor of 17.82[22], presumably

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because of an induced change in the specific growth rate u. In this case

$$z_{(\text{stripped})} = u \times 17.82 = 0.15 \text{ (days)}^{-1} \quad (16)$$

which results in $T = 6.5$ days and $t_d = 4.5$ days.

Similarly, since it is stated that in psoriasis the number of mitoses per unit surface area of the skin is 27 times greater than in normal epidermis[19],

$$z_{(\text{psoriasis})} = u \times 27 = 0.23 \text{ (days)}^{-1} \quad (17)$$

giving $T = 4.3$ days and $t_d = 3.0$ days. In the same publication, a calculation based on different considerations but employing the same data yielded a turnover time of 4 days[19].

Nutritive control. The basic similarity between the cellular population of the epidermis and steady state cultures of micro-organisms enables a tentative application to the epidermis of the quantitative relationships which have been experimentally established for the latter. Thus the belief in the existence of a definitive numerical relationship between the amount of nutrients consumed by the epidermis and the growth of the population of epidermal cells, both expressed per unit area of skin surface, is strengthened by the circumstance that an analogous relationship characterizes microbial steady state cultures, where it was formally expressed by

$$dM/dt = Y dS/dt \quad (18)$$

where dM/dt is the rate of growth, dS/dt the rate of utilization of the limiting substrate, and Y a constant[18]. Similarly, since it appears that nutrient supply affects growth by influencing mitotic activity, use can be made of the finding that the dependence of the specific growth rate u of bacteria on the concentration of the limiting metabolite in the culture medium is closely described by the relation[17,18]

$$u = u_M \frac{S}{K_s + S} \quad (19)$$

where u_M is the maximal specific growth rate obtainable with a given substance, and K_s is a constant. Thus, relating both the rate of nutrient supply and the mitotic activity to a unit surface area of the skin and resolving S into the components S_e and S_d, we obtain for the epidermis the analogous general equation

$$z = z_M \frac{m\,S_e + n\,S_d}{K_s + m\,S_e + n\,S_d} \quad (20)$$

where m and n indicate how much S_e and S_d must be altered in order to account for a given value of z in a given situation.

To illustrate the possible usefulness of this approach, equation (20) is simplified to

$$z = z_M \frac{a\,S}{K_s + a\,S} \quad (21)$$

where

$$a\,S = m\,S_e + n\,S_d \quad (22)$$

Taking for the normal skin, $a = 1$, $z = 0.037$, and $z_M = 0.333$, we obtain $K_s = 8S$, which is assumed to remain constant in abnormal situations as well. The value $z_M = 0.333$ is based on some preliminary observations on the increment of mitotic activity of stripped epidermis cultured in vitro[23] and may be in need of revision.

Applied to psoriatic skin, equation (21) takes the form:

$$0.23 = 0.333\,a\,S / (8\,S + a\,S)$$

from which $a = 18.37$.

Again, for stripped skin, where $z = 0.15$ (days)$^{-1}$ one obtains from equation (21), $a = 6.8$.

Thus, the nutritive flux in psoriasis is about 18 times, and that of stripped skin about 7 times greater than normal. Obviously, this conclusion reflects the data used in the calculations. Further fractionation of the nutritive flux into its diffusional and bulk flow components must await additional information, especially since both in psoriasis and following the destruction of the skin barrier to water by stripping[24], the high evaporational loss of the epidermis may reduce or even abolish the diffusional component of the nutritive flux.

Psoriasis: a barrier disease? The diffusion of metabolites into the epidermis considered per unit area of skin surface ought to be proportional to the surface area of the basement membrane. Thus, tentatively, using the data from a recent study[19] and with the aid of equation (3) the diffusional component of the nutritive flux of psoriatic skin should exceed that of normal skin by a factor of

$$\frac{S_d \text{ (psoriasis)}}{S_d \text{ (normal skin)}} = \frac{A_j/A_s \text{ (psoriasis)}}{A_j/A_s \text{ (normal skin)}} = 2.31 - 4.76 \quad (23)$$

provided the metabolic gradients and diffusion coefficients are the same in both situations. Comparing this result with the total increase of the nutrient flux calculated earlier on the basis of cell production and considering that the bulk-flow-mediated flux may diminish the diffusional transport by affecting the activity gradients, one is led to conclude that in psoriasis a significant portion of the total nutritive flux is due to a heightened evaporative loss from the surface of the skin, and that this is probably due to a defective skin barrier to water. This conclusion suggests that the range of factors which are considered to control the mitotic activity of the epidermis ought to include environmental humidity and wind velocity, which become more important the less efficient the skin barrier. Consequently, occlusive treatment of psoriatic areas alone may significantly reduce the extent of scaling and simulate the situation in normal skin by limiting the mitotic activity to the basal cell layer, but is unlikely to reduce the extensive interdigitations at the dermal-epidermal interface and the diffusional flux across it.

These conclusions are strengthened by the finding that psoriatic areas are characterized by high rates of water loss[25]; attention having been drawn to this factor as possibly involved in the aetiology of this disorder[26]. Further support is derived from clinical observations[27], and from the apparent association of increased incidence and severity of psoriasis with dry as compared to humid geographical regions[28].

However, it is necessary to point out that there are no data available which would suggest the identity of the limiting metabolite which is presumed to control the mitotic activity of the epidermis, nor even to indicate that it is a single substance. In addition, consideration should be given to the possibility that a defective skin barrier to water may also be defective with respect to the

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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.

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[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.

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