[1] K. C. HIGHNAM, Mem. Soc. Endocrin. *12*, 379 (1962).
[2] L. HILL, J. Insect Physiol. *8*, 609 (1962).
[3] K. SLÁMA, J. Insect Physiol. *10*, 773 (1964).
[4] R. R. MILLS, F. C. GREENSLADE and E. F. COUCH, J. Insect Physiol. *12*, 767 (1966).
[5] E. ANDERSON, J. Cell Biol. *20*, 131 (1964).
[6] T. F. ROTH and K. R. PORTER, J. Cell Biol. *20*, 313 (1964).
[7] F. ENGELMANN and D. PENNEY, Gen. comp. Endocrin. *7*, 314 (1966).
[8] K. C. HIGHNAM, in *Insect Reproduction*, Symp. Soc. R. Entomol. Soc. London (1964), vol. 2.
[9] F. ENGELMANN, A. Rev. Ent. *13*, 1 (1968).
[10] K. C. HIGHNAM, O. LUSIS and L. HILL, J. Insect Physiol. *9*, 587 (1963).
[11] K. C. HIGHNAM, O. LUSIS and L. HILL, J. Insect Physiol. *9*, 827 (1963).
[12] A. G. GORNALL, C. J. BARDAVILL and M. M. DAVID, J. biol. Chem. *177*, 751 (1949).
[13] A. G. E. PEARSE, *Theoretical and Applied Histochemistry* (J. and A. Churchill, London 1968), vol. 1.
[14] We thank Prof. K. K. NAYAR for critically going through the manuscript, the Ford Foundation for a generous grant and one of us (M.J.) the University of Kerala for a Junior Research Fellowship.

of day 1 showed that all the excess proteins were apparently utilized by the growing oocytes. Inspite of low blood protein concentration, vitellogenesis continued on day 4. Vitellogenesis failed to occur till day 2 not because of the absence of any vitellogenic proteins in the blood. Thus it appeared that protein build-up in the blood on the one hand, and its deposition as yolk in the oocytes on the other, were controlled by 2 factors in *Dysdercus cingulatus*: the former probably by neurosecretion from the brain and the latter by the corpus allatum hormone, as in *Schistocerca*[2,8,10,11].

*Zusammenfassung.* Nachweis, dass Blutproteine bei der Wanze *Dysdercus cingulatus* die Konzentration am 2. Tag nach der Eiabstossung verdoppeln, ohne dass Eiweissdotter in den Oozyten auftritt.

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## Microsomal Phospholipid Biosynthesis after Phenobarbital Administration

Hypertrophy of the endoplasmic smooth membranes in the hepatocyte is a common response to the microsomal enzyme induction after administration of a large number of drugs and pesticides[1,2].

The mechanism of the phospholipid-rich lipoprotein matrix overgrowth is unclear. Previous reports on the microsomal phospholipid synthesis, an indicator of the lipoprotein turnover, are conflicting. ORRENIUS et al.[3,4] found an increase of phospholipid synthesis in the liver microsomal membranes of rats treated with phenobarbital. Using the same $^{32}P$-labelling technique. HOLTZMAN et al.[5] recently drew the conclusion that after phenobarbital treatment the synthesis of membrane phospholipids is not increased and thus the membrane hypertrophy is due to a slower catabolism. It is difficult to settle the problem because the label of tissue phospholipid in vivo after a pulse of radiophosphorus measures only the complete synthesis of phospholipid molecules from the terminal phosphate group of ATP and the 1,2-diglyceride[6]. Two other metabolic pathways, conversion of lysophospholipids to phospholipids[7] and the lecithin synthesis by successive methylations of phosphatidyl ethanolamine[8] are not evaluated by the $^{32}P$ phosphate incorporation. These two pathways are active in the endoplasmic reticulum.

The present experiment studies the behaviour of these different pathways in the rat liver microsomes by simultaneous labelling with $^{32}P$-phosphate and $^{14}C$-fatty acid after a single administration of phenobarbital.

*Material and methods.* 12 male Sprague Dawley rats (Charles River, C.D.), 200–210 g body weight, were given 80 mg/kg of phenobarbital (Merck) i.p. Controls received 1 ml of saline. All the animals were fasted and allowed to drink 20% (W/V) glucose for 24 h. After that time each animal received i.v. 0.5 mc of $^{32}P$-phosphate, spec. act.: 20 μci/μg and 2 μci of $^{14}C$-palmitic acid, spec. act.: 50 mci/m*M* (C.E.A. Saclay, France). Rats were exsanguinated 1 h later under slight ether anesthesia. Blood was collected on heparine and centrifuged in the cold. Livers were rapidly scissed, blotted and weighed. 1 g of tissue was homogeneized with 10 ml of chilled 5% trichloroacetic acid. After centrifugation, soluble phosphorus[9] and radioactivity were measured in the supernatant. Aliquots were used for P determination[9] and radioactivity counting. Microsomal membranes were isolated[10] and aliquots were used for anilinhydroxylase[10] and protein[11] determinations. Lipids were extracted by the FOLCH's technic[12] and lipid phosphorus was measured[9].

Phospholipids were separated by thin layer chromatography (Silicagel G Merck). The spots of individual phospholipids marked after exposure to iodine vapours were scraped off into counting vials. After 10 min at 100 °C in the oven, the vials were dried under vacuum, then 10 ml of scintillating solution (Toluene, PPO, POPOP) were added; the $^{14}C$ and $^{32}P$ radioactivity was counted 3 h later in a Mark II spectrometer (Nuclear Chicago) in channels set up for $^{32}P$ and $^{14}C$ respectively.

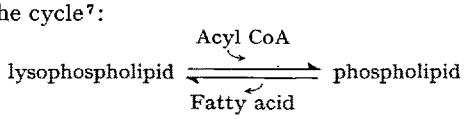
*Results and discussion.* After a single phenobarbital dose the protein and the total phospholipid contents of the microsomal fraction were increased (Figure 1). Furthermore anilin hydroxylase activity increases in accordance with previous observations[5]. Repeated administrations

[1] H. REMMER and H. J. MERKER, Ann. N.Y. Acad. Sci. *123*, 79 (1963).
[2] A. H. CONNEY, Pharmac. Rev. *19*, 317 (1967).
[3] S. ORRENIUS, J. L. E. ERICSSON and L. ERNSTER, J. Cell. Biol. *25*, 627 (1965).
[4] S. ORRENIUS and J. L. E. ERICSSON, J. Cell. Biol. *28*, 181 (1966).
[5] J. L. HOLTZMAN and J. R. GILLETTE, J. biol. Chem. *243*, 3020 (1968).
[6] E. P. KENNEDY and S. B. WEISS, J. Am. chem. Soc. *77*, 250 (1955).
[7] W. E. M. LANDS, J. biol. Chem. *235*, 2233 (1960).
[8] G. BREMER and D. M. GRENBERG, Biochim. biophys. Acta *70*, 389 (1963).
[9] J. L. DELSAL and H. MANHOURI, Bull. Soc. Chim. biol. *37*, 1041 (1955).
[10] R. KATO and J. R. GILLETTE, J. Pharmac. exp. Ther. *150*, 279 (1965).
[11] O. H. LOWRY, N. J. ROSENBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[12] J. FOLCH, M. LEES and G. H. SLOANE-STANLEY, J. biol. Chem. *226*, 19 (1957).

of the drug gradually enhance both the anilin hydroxylase activity and the microsomal protein and phospholipid contents.

As Figure 2 shows the radioactivity incorporated into microsomal phospholipids expressed in dpm per μg of microsomal protein is increased both for $^{32}P$ and $^{14}C$ suggesting an overall increase of the membrane phospholipid synthesis as previous reports indicated[3,4]. Moreover, the specific activity of phospholipids (dpm/μg of P) significantly increases in the treated animals (Table I). Phenobarbital administration modifies the phosphate pool for some unknown reason. Thus the specific activity of intracellular phosphate is higher in the drug-treated group than in the controls. So the relative specific activity, i.e. the ratio of specific activity of phospholipids to the specific activity of intracellular phosphate, is identical in both groups. Obviously the synthesis rate of the total microsomal phospholipids through the KENNEDY's pathway[6] is not increased under phenobarbital treatment. The same conclusion has been drawn for lecithin and phosphatidylethanolamine, the 2 major phospholipids in the endoplasmic membranes.

If the cycle[7]:




had been enhanced, more $^{14}C$ palmitic acid should have been incorporated into the microsomal phospholipid. However this is not the case, so we can reasonably conclude, since $^{14}C$ palmitate uptake by the liver and the specific activity of the homogenate $^{14}C$ palmitic acid are not different from the controls, that the Land's pathway is not modified by the drug treatment.

The conversion of phosphatidylethanolamine into lecithin[8] cannot be directly estimated with our labelling techniques. Nevertheless we can take into account that, though the concentration of both phospholipids is increased in the microsomes of phenobarbital treated rats, relatively more label can be recovered in the phosphatidylethanolamine from phenobarbital group (data not shown) suggesting that its conversion into lecithin may have been slown.

Finally the plasma phospholipids are labelled to a lesser extent in the phenobarbital treated rats (Table II). Correction of the phospholipids specific activity for the specific activity of the $^{32}P$ inorganic precursor gives sharper results, indicating that the synthesis rate or the release of plasma phospholipids is decreased. The slight increase in the concentration suggests that the plasma phospholipid catabolism was slown down in the drug-treated rats.

In conclusion our results support the view that drug-induced endoplasmic membranes hypertrophy is promoted by an inhibition of the liver phospholipid catabolism more than in an enhanced synthesis. Furthermore our

Table I. Incorporation of $^{32}P$-radioactivity in the microsomal phospholipids and in the liver acid-soluble phosphorus

| | Phospholipids specific activity (dpm/μg of P.) | P. acid-soluble specific activity (dpm/μg of P.) | S. activity PL × $10^3$ / S. activity P. ac. sol. |
|---|---|---|---|
| Control | 787 ± 62 | 23,800 | 33.1 |
| Phenobarbital | 1,061 ± 87 | 30,600 | 33.7 |

Mean ± S.D. of 12 rats per group.

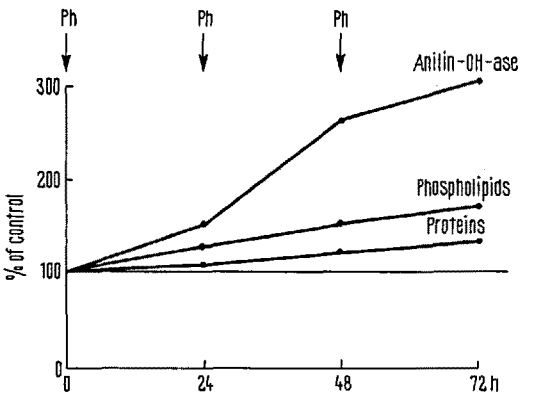



Fig. 1. Effect of repeated injections of phenobarbital (80 mg/kg) on the liver microsomal protein, phospholipids and anilinhydroxylase activity. Average values of 8 to 12 rats per group.

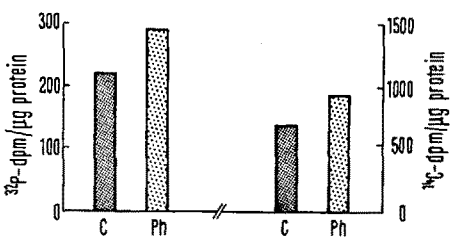



Fig. 2. Incorporation of $^{32}P$-phosphate and $^{14}C$-palmitic acid in the microsomal phospholipid. Average values of 12 rats per group. Phospholipid radioactivity is expressed in dpm per μg of microsomal protein.

Table II. Incorporation of $^{32}P$-radioactivity in the plasma phospholipids

| | Lipid P (μg/ml) | $^{32}P$-radioactivity dpm/ml plasma | P lipid S.A. dpm/μg of P. | S. activity of plasma PL × $10^4$ / S. activity of liver P. acid sol. |
|---|---|---|---|---|
| Control | 87 ± 5 | 2,980 ± 154 | 34.3 ± 4 | 14.9 |
| Phenobarbital | 97 ± 7 | 2,305 ± 180 | 19.1 ± 5 | 6.2 |

Mean ± S.D. of 12 rats per group.

data indicate that plasma phospholipids turnover is also retarded.

After these experiments were completed, a paper from STEIN and STEIN[13] showed direct experimental evidence that microsomal phospholipase activity is depressed in the rat liver after phenobarbital treatment, confirming the data of turnover rate of HOLTZMAN[5] and our own results, in the sense of a decrease in the phospholipid catabolism[14].

*Résumé.* Après administration de phénobarbital chez le rat le renouvellement des phospholipides microsomiaux étudiés par double marquage $^{14}C$-$^{32}P$ n'est pas augmenté alors que celui des phospholipides plasmatiques est ralenti. L'hypertrophie des membranes endoplasmiques n'est pas le résultat d'une augmentation de leur biosynthèse et semble être la conséquence d'un ralentissement du catabolisme.

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[13] Y. STEIN and O. STEIN, Israel J. med. Sci. *5*, 985 (1969).

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## Mechanism of Action of Boseimycin

Boseimycin[1,2], a new streptothricin-like antibiotic, was isolated from culture broth of an unidentified *Streptomyces* sp $Ac_6$ 569. It is effective against a number of gram positive and gram negative bacteria, fungi and yeast in vitro. The mechanism of action of boseimycin, investigated with *Bacillus subtilis*, is reported here.

*Experimental.* A sterile aqueous solution of boseimycin hydrochloride was obtained by membrane filtration. Nutrient medium containing bacto peptone, 5 g/l; beef extract (Difco) 10 g/l and NaCl, 5 g/l in distilled water was used in all growth experiments excepting those with radioactive precursors, when a semisynthetic medium containing peptone, 2.5 g/l; NaCl, 5 g/l and glucose 5 g/l in distilled water was used. The pH of the media was maintained at 7.4 before sterilisation.

For growth studies, the culture grown overnight was diluted 20-fold with fresh medium. Liquid cultures were incubated at 37 °C by agitation. The cells were harvested in logarithmic growth phase (turbidity, 0.5 at 600 nm), centrifuged below 4 °C, washed twice with 0.05 *M* phosphate buffer and resuspended in liquid medium. The cells were incubated for 90 min in the medium prior to antibiotic addition. Absorbancy was measured at 600 nm with a Bosch and Lomb Spectronic colorimeter. Viable cell counts were obtained by use of a spreading plate method on nutrient agar.

Nucleic acids and protein syntheses were determined on 10 ml aliquots removed at required intervals. DNA, RNA and protein fractions were obtained after a modified SCHMIDT-THANHAUSSER[3] method and estimated colorimetrically by standard diphenylamine[4], orcinol[5] and LOWRY's[6] methods respectively.

Cells at early logarithmic phase were used for the incorporation of $^{14}C$-leucine and carrier free $^{32}P$-phosphoric acid as precursors for the syntheses of cellular protein and nucleic acids. Aliquots were taken at intervals and the reaction was stopped with ice-cold 5% perchloric acid. The protein and nucleic acids were fractionated as above and the rate of incorporation was expressed as cpm per ml of the suspended culture.

*Results and discussion.* The effect of boseimycin on growth and macromolecular syntheses of *B. subtilis* are shown in Figures 1 and 2. In the presence of 0.2 µg/ml of the antibiotic, the turbidity of the culture continued to increase but at a slightly lower rate than that of the control. Macromolecular syntheses paralleled to the growth of bacteria. On the other hand, an immediate cessation of bacterial growth was observed after the addition of the antibiotic at 2 µg/ml. Protein synthesis was concurrently inhibited, which could be correlated with the growth inhibition. DNA and RNA syntheses were levelled off comparatively at a later stage. Similar results were observed in another set of experiments where

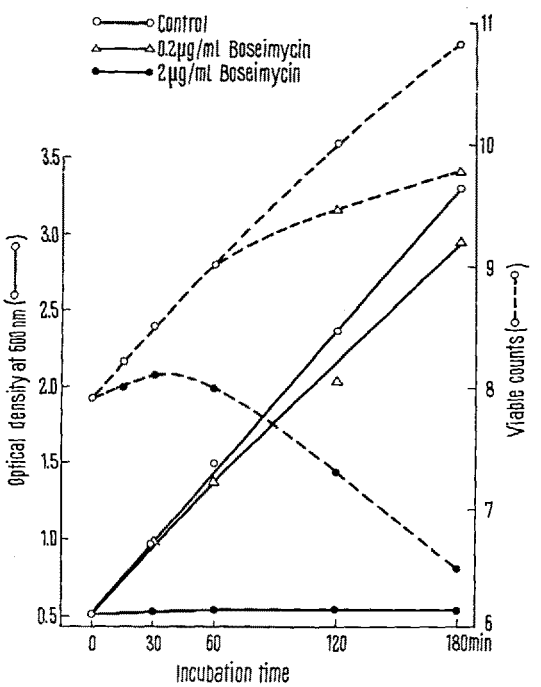


Fig. 1. Effect of boseimycin on the growth of *B. subtilis*. Boseimycin was added after 90 min of incubation under agitation. The dotted and solid lines represent changes in the viable cell counts and optical density respectively.

[1] R. K. SINHA and P. N. NANDI, Experientia *24*, 795 (1968).

[2] R. K. SINHA, J. Antibiotics, Tokyo *23*, 360 (1970).

[3] G. SCHMIDT and S. J. THANHAUSSER, J. biol. Chem. *161*, 83 (1945).

[4] K. BURTON, Biochem. J. *62*, 315 (1956).

[5] G. ASHWELL, *Methods in Enzymology* (Academic Press Inc., New York 1957), vol. 3, p. 87.

[6] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).