The Influence of Phenobarbital on the Glycogen Concentration of Rat Liver

It is already known that pentobarbital changes the level of glycogen in the liver of experimental animals[1,2]. The relation of phenobarbital towards the glycogen metabolism has remained insufficiently determined until the present day. Thus, it was discovered that smooth endoplasmatic reticulum hypertrophy in the liver of phenobarbital-treated rats is followed by the lack of monoparticulate glycogen[3]. However, REMMER and MERKER[4] could not find, by biochemical methods, that total glycogen decreases in the liver of phenobarbital-treated rats.

In the present study data on variation and relation of free and bound glycogen in the liver of phenobarbital-treated rats are given. The results described below show that phenobarbital changes the level of free and bound glycogen in the liver of phenobarbital treated rats.

Material and methods. Experiments were carried out on male albino rats, weighing between 120–150 g. In the first experimental group we examined the effect of a single phenobarbital injection on the liver glycogen content. The animals were killed in groups 20 min, 3 and 24 h after the administration of the first phenobarbital injection. In the second and third experimental groups, the effect of the repeated administration of phenobarbital (5 and 15 injections) was examined. The control groups received an adequate quantity of saline solution. Phenobarbital was injected by i.p. way in daily doses of 100 mg/kg of body weight. All the animals were fasted 24 h prior to killing, in order to exclude the variations of individual food consumption. By measuring the food consumption daily throughout the duration of experiments, we found that there is no difference in the amount of food eaten by the phenobarbital-treated rats as compared with the saline-injected one.

The animals were decapitated between 8.00 and 9.00 a.m. Free (readily extracted) and bound (residual) glycogen were determined in the liver samples according to the method of MONTGOMERY[5]. The total glycogen is expressed as the sum of free and bound glycogen. Blood sugar was determined according to the method of HAGEDORN and JENSEN[6].

Results. Table I shows the effect of the single phenobarbital dose on the glycogen level in the rat liver. The liver glycogen level changes only 24 h after the administration of one phenobarbital dose. Free glycogen decreases by 34% ($p < 0.01$). The decrease of bound glycogen is not statistically significant. Total glycogen decreases by 22.6% ($p < 0.05$). Five days treatment of animals with phenobarbital (Table II) leads to a decrease of free glycogen by 36% ($p < 0.01$), as well as the bound glycogen by 46% ($p < 0.01$). Fifteen days phenobarbital treatment of animals causes the decrease in the concentration of free glycogen by 28% ($p < 0.01$) as well as bound liver glycogen by 27% ($p < 0.01$). Phenobarbital has no effect on the level of blood sugar.

Discussion. It is well known that phenobarbital treatment brings about great alterations in the biochemical and structural composition of the parenchymal liver cells: it increases activity of drug metabolizing enzymes[4], lipid, RNA and protein quantity[7] and induces hypertrophy and hyperplasia of parenchymal liver cells[8]. The results of our experiments presented here show that phenobarbi-

[1] P. DESBALS, C. r. Acad. Sci. *159*, 880 (1965).
[2] R. AGID and P. MIAHLE, C. r. Acad. Sci. *248*, 2902 (1959).
[3] J. R. FOUTS, Fedn Proc. *21*, 1107 (1962).
[4] H. REMMER and H. J. MERKER, Science *142*, 1657 (1963).
[5] R. MONTGOMERY, Arch. Biochem. biophys. *67*, 378 (1957).
[6] H. C. HAGEDORN and B. N. JENSEN, Biochem. Z. *92*, 137 (1923).
[7] S. ORRENIUS, J. L. E. ERICSSON and L. ERNSTER, J. Cell Biol. *25*, 627 (1965).
[8] M. JAPUNDŽIC, B. KNEŽEVIĆ, V. DJORDJEVIĆ-ČAMBA and I. JAPUNDŽIĆ, Expl Cell Res. *48*, 163 (1967).

Table I. The influence of one injection of phenobarbital-Na on liver glycogen content in the rats

Time after one injection	No. of experiments	Free glycogen	% of decrease	p value	Bound glycogen	% of decrease	p value	Total glycogen	% of decrease	p value
20 min	8	12.4 ± 3.05	12.0	> 0.05	34.5 ± 7.84	7.2	> 0.05	46.9 ± 8.89	8.5	> 0.05
3 h	8	11.4 ± 2.69	18.5	> 0.05	36.7 ± 3.75	1.3	> 0.05	48.1 ± 5.07	6.0	> 0.05
24 h	10	9.2 ± 2.58	34.0	< 0.01	30.4 ± 6.51	18.2	> 0.05	39.6 ± 5.92	22.6	< 0.05
Controls	10	14.0 ± 3.56			37.2 ± 11.68			51.2 ± 11.68		

Table II. The influence of repeated administration of phenobarbital-Na on liver glycogen content in the rats

	No. of experiments	Free glycogen	% of decrease	p value	Bound glycogen	% of decrease	p value	Total glycogen	% of decrease	p value
After 5 injections	6	11.70 ± 2.49	36.0	< 0.01	22.90 ± 5.35	46.0	< 0.01	34.60 ± 7.58	41.0	< 0.01
After 15 injections	8	13.20 ± 2.74	28.0	< 0.01	30.80 ± 7.55	27.0	< 0.01	44.00 ± 9.82	24.0	< 0.01
Controls	8	18.30 ± 4.78			42.20 ± 5.72			58.00 ± 9.25		

tal causes the fall in the concentration of free, bound and total glycogen content in rat liver. The question is raised concerning the relationship between the fall in glycogen concentration and the above-mentioned complex of biochemical[4,7-9] and structural events[4] which follow phenobarbital treatment. First of all, this question is related to the increased activity of drug metabolizing enzymes[4]. It was found that the activity of drug metabolizing enzymes in parenchymal liver celles, under certain experimental conditions is parallel to the quantity of liver glycogen[10-12]. That is why FOUTS et al.[13] have postulated that there is correlated dependence between the glycogen quantity and the activity of drug metabolizing enzymes. On the contrary, data of WOOLES[14] does not support the concept of FOUTS et al.[13]. Analyzing the influence of prolonged phenobarbital treatment on the activity of drug metabolizing enzymes, ORRENIUS[7] has found that its activity is increased progressively until the fifth day of treatment. If our results are compared with the results of ORRENIUS[7], the conclusion is reached that there is no direct dependence between the level of glycogen and the degree of activity of drug metabolizing enzymes in the parenchymal liver cells of phenobarbital treated animals. However, we cannot exclude the possibility that the observed decrease in glycogen concentration is at least partly the consequence of increased synthesis of liver microsomal enzymes.

On the other hand, the growth-promoting effect of phenobarbital can also be related to the above-mentioned decrease of glycogen concentration. It is already known that intensification of the proliferative power of tissues in the case of cancerization[15] as well as in the case of normal regeneration of the liver[16,17] is always followed by a decrease in glycogen concentration. The same antiparallel relationship between the quantity of glycogen and mitotic activity of the liver is evident from the present and also our earlier published observations[8,18].

Résumé. On a constaté qu'une injection du phénobarbital provoque la diminution de la concentration du glycogène libre de 34% et la diminution du glycogène total de 22,6% seulement 24 h après leur injection. La répétition des injections du phénobarbital diminue le glycogène libre respectivement de 36% et de 28%, le glycogène lié de 46% et de 27%, et le glycogène total de 41% et 24%.

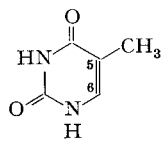
I. JAPUNDŽIĆ, M. JAPUNDŽIĆ, B. KNEŽEVIĆ and J. MIMIĆ-OKA

Biochemical Institute and Histological Institute, Medical Faculty, Belgrade (Yugoslavia),
13 August 1968.

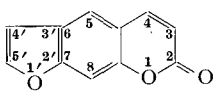
[9] T. S. ARGYRIS and D. R. MAGNUS, Devl. Biol. *17*, 187 (1968).
[10] R. L. DIXON, R. W. SHULTICE and J. R. FOUTS, Proc. Soc. exp. Biol. Med. *103*, 333 (1960).
[11] R. L. DIXON, L. G. HART and J. R. FOUTS, Pharmac. exp. Ther. *7*, 133 (1961).
[12] J. R. FOUTS, R. L. DIXON and R. W. SHULTICE, Biochem. Pharmac. *7*, 265, 1961.
[13] L. H. ROGERS, R. L. DIXON and J. R. FOUTS, Biochem. Pharmac. *12*, 341 (1963).
[14] W. R. WOOLES and J. J. MCPHILLIPS, Proc. Soc. exp. Biol. Med. *121*, 399 (1966).
[15] K. R. PORTER and C. BRUNI, Cancer Res. *19*, 997 (1959).
[16] R. J. STENGER and D. B. CONFER, Expl molec. Path. *5*, 455 (1966).
[17] A. H. CONNEY and J. J. BURNS, Adv. Pharmac. *1*, 31 (1962).
[18] Supported by a grant from Z.M.N.U.-S.R.S.

A Comparison Between the Photoreactivity of Some Furocoumarins with Native DNA and Their Skin-Photosensitizing Activity

Some years ago we found that photosensitizing furocoumarins give a photoreaction with nucleic acids by irradiation at 365 nm[1,2]. A C_4-cyclo-addition reaction of furocoumarins to the pyrimidine bases of the macromolecules takes place. Pyrimidine bases always react with their 5,6-double bond and furocoumarins may react either with their 3,4 or with their 4',5' double bond[3-6].



Thymine



Psoralen

This photoreaction appears to explain the mechanism of the photosensitizing action that furocoumarins exert on bacteria[7-9], on mammalian cells in vitro grown[10], on DNA-viruses[11] on mouse Ehrlich ascites tumour cells[12], on sea-urchin sperm[13], and also their skin-photosensitizing activity[14-17] (outcome of erythema on human and guinea-pig skin after a latent period), even if at present the connection between the damage to DNA and the outcome of the erythema is not clear.

In order to test this connection, we have evaluated the photoreactivities of a number of furocoumarins with native DNA by irradiation at 365 nm and we have com-

[1] L. MUSAJO, G. RODIGHIERO and F. DALL'ACQUA, Experientia *21*, 24 (1965).
[2] L. MUSAJO, G. RODIGHIERO, A. BRECCIA, F. DALL'ACQUA and G. MALESANI, Photochem. Photobiol. *5*, 739 (1966).
[3] L. MUSAJO, F. BORDIN, G. CAPORALE, S. MARCIANI and G. RIGATTI, Photochem. Photobiol. *6*, 711 (1967).
[4] L. MUSAJO, F. BORDIN, R. BEVILACQUA, Photochem. Photobiol. *6*, 927 (1967).
[5] L. MUSAJO, G. RODIGHIERO, F. DALL'ACQUA, F. BORDIN, S. MARCIANI and R. BEVILACQUA, Rc. Accad. naz. Lincei *42*, 457 (1967).
[6] C. H. KRAUCH, D. M. KRÄMER and A. WACKER, Photochem. Photobiol. *6*, 341 (1967).
[7] W. L. FOWLKS, D. G. GRIFFITH and E. L. OGINSKY, Nature *181*, 571 (1958).
[8] E. L. OGINSKY, G. S. GREEN, D. G. GRIFFITH and W. L. FOWLKS, J. Bact. *78*, 821 (1959).
[9] M. M. MATHEWS, J. Bact. *85*, 322 (1963).
[10] G. COLOMBO and A. G. LEWIS, Progr. Biochem. Pharmac. *1*, 392 (1965).
[11] L. MUSAJO, G. RODIGHIERO, G. COLOMBO, V. TORLONE and F. DALL'ACQUA, Experientia *21*, 22 (1965).
[12] L. MUSAJO, P. VISENTINI, F. BACCICHETTI and M. A. RAZZI, Experientia *23*, 335 (1967).
[13] G. COLOMBO, Expl Cell Res. *48*, 168 (1967).
[14] L. MUSAJO, G. RODIGHIERO and G. CAPORALE, Bull. Soc. chim. Biol. *36*, 1213 (1954).
[15] L. MUSAJO and G. RODIGHIERO, Experientia *18*, 153 (1962).
[16] M. A. PATHAK, J. H. FELLMAN and K. D. KAUFMAN, J. invest. Derm. *35*, 165 (1960).
[17] M. A. PATHAK, L. R. WORDEN and K. D. KOUFMAN, J. invest. Derm. *48*, 103 (1967).