indicate an increase in the rate of turnover of mRNA and of heterogeneous nRNA. In the case of heterogeneous nRNA, by the 24th week of the experiment the increase in the rate of incorporation of 5-^{3}H-uridine compared with normal was considerable (Fig. 2a-f). As the results of correlation analysis show, from the 12th to the 24th weeks of the experiment synchronization of metabolism between individual fractions of heterogeneous nRNA also was disturbed. The results described above are evidence of profound changes in transcription in the liver as a result of chronic CCl_4 poisoning.

LITERATURE CITED

1. L. A. Voronova, S. D. Ivanov, and M. A. Zabezhinskii, Byull. Éksp. Biol. Med., 81, No. 5, 543 (1976).
2. S. D. Ivanov, Abstracts of Proceedings of a Conference of Junior Scientific Oncologists of Leningrad Research Institute of Oncology [in Russian], Leningrad (1974), p. 36.
3. S. D. Ivanov, L. A. Voronova, M. A. Zabezhinskii, et al., Vopr. Onkol., 20, No. 8, 54 (1974).
4. S. D. Ivanov, Yu. P. Zerov, L. A. Voronova, et al., Vopr. Onkol., No. 6, 84 (1974).
5. N. A. Plokhinskii, Biometrics [in Russian], Moscow (1970).
6. A. Fisher, The Physiology and Experimental Pathology of the Liver [in Russian], Budapest (1961).
7. J. Hoffman, M. B. Himes, S. Lapan, et al., Arch. Pathol. 59, 429 (1955).
8. H. J. Seitz and W. Tarnowski Hoppe-Seylers Z. Physiol. Chem., 349, 1800 (1968).
9. F. Verney, C. N. Murty, and H. Sidransky, Fed. Proc., 35, 551 (1976).
10. R. A. Weinberg, Ann. Rev. Biochem., 42, 329 (1973).

INVESTIGATION OF THE POSSIBLE USE OF IMMOBILIZED UREASE FOR DECOMPOSING UREA IN BLOOD PLASMA

I. P. Mel'nik, M. N. Molodenkov, O. A. Mashkov, Yu. V. Artemova, R. I. Fesenko, A. D. Virnik, and V. Ya. Yakovlev

UDC 612.124:612.398.193

The rate of decomposition of urea in citrated donors' plasma by soluble urease and by urease immobilized by addition to the carboxymethyl ester of cellulose, the 2-(3'-amino-4'-methoxyphenyl)sulfonylethyl ester of cellulose, the diethylaminoethyl ester of cellulose, stained with dichlorotriazine dye, or the grafted copolymer of cellulose and polyglycidylmethacrylate was found to be closely similar. Preparations of immobilized urease can be used repeatedly to decompose urea in citrated donors' plasma. Periodic treatment of these preparations with cysteine solution resulted in a smaller degree of decrease in the enzyme activity of the immobilized urease during repeated use.

KEY WORDS: *urea, urease; immobilization of enzymes; blood plasma.*

A basically new method of purifying blood from toxic substances has begun to be developed in recent years, namely the hemosorption method. This method is based on the use of "sorbents," capable of selectively removing certain toxic substances from the blood, or specific catalysts which convert toxic into less toxic substances. In 1971, Lopukhin et al.

N. I. Pirogov Second Moscow Medical Institute. Moscow Textile Institute. All-Union Vitamin Scientific-Research Institute, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 4, pp. 425-427, April, 1977. Original article submiteed October 22, 1976.

TABLE 1. Data Showing Possibility of Repeated Use of Immobilized Urease Preparations for Decomposing Urea in Blood Plasma*

Cellulose derivatives used to immobilize urease	Duration of incubation	Quantity of urea hydrolyzed, % cycle																									
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
CMC	90	37	44	40	33	36	34	34	34	34	21	14	16	18†	34	7	7	7	2,5	—	—	—	—	—	—	—	—
	150	100	72	65	44	48	45	43	32	34	20	8	8	—	—	—	—	—	—	—	—	—	—	—	—	—	—
DEAC-CTD	120	62	45	41	46	—	30	39	32	49	37	51	35	48	35	54	33	53	31	48	27	36	—	40	—	50	30
	120‡	62	50	59	53	48	48	46	47	49	51	51	51	55	48	55	48	48	—	47	37	40	35	40	36	54	40

*4 ml blood plasma, urea concentration 8 mg/ml, activity of enzyme preparation 1 unit.

†After 13th cycle preparation of immobilized urease was treated with 0.001 M cysteine solution for 12 h.

‡After each cycle preparation of immobilized urease was treated with 0.001 M cysteine solution for 1 min.

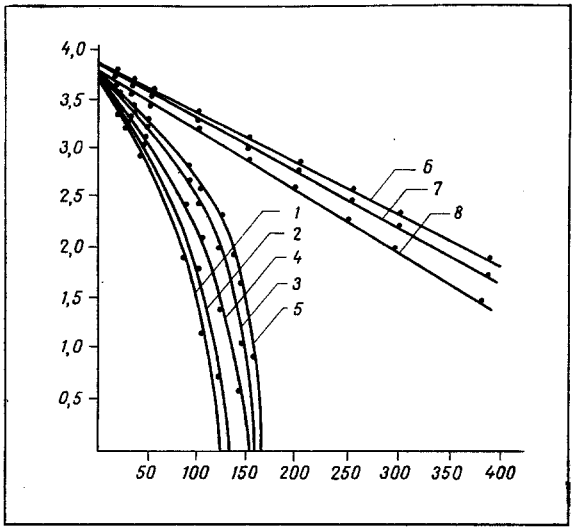

Fig. 1. Degree of decomposition of urea by soluble and immobilized urease as a function of time. Abscissa, time, min; ordinate, concentration of urea nitrogen, mg/ml. 1) Soluble urease (1 unit), citrated donors' plasma. 2) Urease immobilized on CMC (1 unit), citrated donors' plasma; 3) urease immobilized on AC (1 unit), citrated donors' plasma; 4) urease immobilized on DEAC-CTD (1 unit), citrated donors' plasma; 5) urease immobilized on C-PGMA (1 unit), citrated donors' plasma; 6) soluble urease (0.24 unit), citrated donors' plasma; 7) soluble urease (0.24 unit), plasma from dog's blood containing heparin; 8) urease immobilized on DEAC-CTD (0.24 unit), citrated donors' plasma.

reported the use of hemosorption under experimental and clinical conditions in the USSR. Among the problems arising during the production of apparatus for hemosorption, one of particular interest is the possibility of using small columns containing the immobilized enzyme urease to reduce the urea concentration *in vivo*. Two methods can be used for this purpose: 1) perfusion of blood through a polymer material containing immobilized urease;

2) separation of the blood cells from the plasma, subsequent decomposition of the urea in the plasma by immobilized urease, and mixing the plasma with the blood cells again.

In this investigation the possibility of using preparations of urease immobilized on various cellulose derivatives, the synthesis of which was described previously [2], to decompose urea in blood plasma was studied.

EXPERIMENTAL METHOD

Soluble urease with an activity of 940 units/g (the number of milligrams of ammonia nitrogen formed during the decomposition of a 2% solution of urea in 0.02 M phosphate buffer, pH 7.0, in the course of 5 min at 20°C by 1 g of enzyme [3] was taken as the unit of activity), and also preparations of urease immobilized on the carboxymethyl ester of cellulose (CMC), the 2-(3'-amino-4'-methoxyphenyl)sulfonylethyl ester of cellulose (AC), the diethylaminoethyl ester of cellulose stained with active dichlorotriazine dye (DEAC-CTD), and a grafted copolymer of cellulose with polyglycidylmethacrylate (C-PGMA) were used. The investigation was carried out under steady-state conditions by the following method. To 4 ml of blood plasma containing about 8 mg/ml of urea 0.5 ml of a solution of urease in 0.1 M phosphate buffer was added, or a suspension of a preparation of immobilized urease in 0.5 ml of 0.1 M phosphate buffer was added; the solution of urease and the preparation of immobilized urease in these cases had equal enzyme activity. The reaction mixture was stirred with a magnetic mixer for a specified period of time at 20°C, after which 0.5 ml of 1 N hydrochloric acid was added to stop the reaction. When immobilized urease was used the mixture was then centrifuged. In both cases the concentration of urea remaining in the solution was then determined by means of the SMA (type 6/60) analyzer. To study the possibility of repeated use of the immobilized urease preparations for reducing the urea concentration in citrated donors' plasma, after the reaction had been in progress for a specified time the preparation of immobilized urease was separated from the plasma by centrifugation and added to a fresh portion of plasma. The urea concentration was determined in each portion of plasma after centrifugation.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1, curves 1-5, that after addition of the enzyme preparation with an activity of 1 unit to the reaction mixture the urea in 4 ml of plasma was completely hydrolyzed in 120-170 min, and that the soluble urease and urease immobilized on CMC, AC, DEAC-CTD, and C-PGMA hydrolyzed urea at almost the same rate. A similar pattern also was observed when preparations with an activity of 0.24 unit were added to the reaction mixture (Fig. 1, curves 6 and 8). The fact that the enzymic activity of the urease was virtually unchanged in the presence of heparin (Fig. 1, curves 6 and 7) is of considerable importance, for under real conditions, when immobilized urease is used, heparin must be added to the patient's blood.

As Table 1 shows, urease immobilized on DEAC-CTD was more stable during repeated use than urease immobilized on CMC. Treatment of the urease preparation immobilized on DEAC-CTD with 0.001 M cysteine solution after each cycle led to a smaller decrease in the enzymic activity of the immobilized urease.

LITERATURE CITED

1. Yu. M. Lopukhin, O. A. Mashkov, K. P. Krylov, et al., Éksp. Khir., No. 4, 73 (1971).
2. R. I. Fesenko, Yu. V. Artemova, A. D. Virnik, et al., Zh. Vses. Khim. O-va, 19, 594 (1974).
3. G. B. Kristiakowsky, P. C. Mangelsdorf, A. J. Resenberg, et al., J. Am. Chem. Soc., 74, 5015 (1952).