of block duration as shown in Figure 1. They exceed the average values of hepatectomized control rats without HU infusion by factors up to about 7.

Previous studies on the degree of synchrony of DNA synthesis after partial hepatectomy revealed that hepatocytes start DNA synthesis in a topographically determined sequence[4]. At 20 to 24 h after operation, the maximum of labelled hepatocytes 60 min after injection of tritiated thymidine is found in the periportal zone of the liver lobule, at 34 h in the intermediary zone, and at 40 h after hepatectomy in parts surrounding the hepatic vein[4]. ^{3}H-thymidine autoradiographs prepared during continuous infusion of HU and at different intervals after termination of HU infusion show that hepatocytes are accumulated at the G_1-S boundary and start, after release from HU block, DNA synthesis with a considerably higher degree of synchrony as compared with normal regenerating liver. An example is given in Figure 2. HU infusion from 14 to 40 h after partial hepatectomy and sacrifice of the rat at 45 h after operation, 60 min after injection of tritiated thymidine, results in a labelling index of 90%. Only a small fringe of tissue around terminal hepatic venules contains unlabelled hepatocytes.

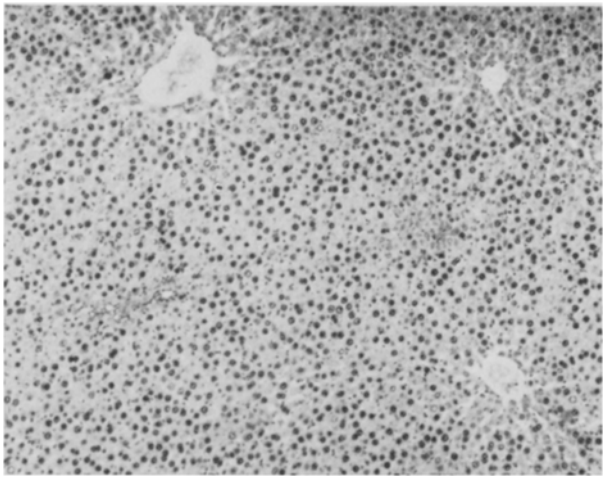

Fig. 2. Increased synchrony of hepatocellular DNA synthesis after release from hydroxyurea block. Autoradiogram of rat liver after continuous infusion of HU (1.25×10^{-3} M kg^{-1} h^{-1}) from 14 to 40 h after partial hepatectomy. Injection of ^{3}H-thymidine (0.8 μCi g^{-1}) at 44 h, sacrifice of the rat at 45 h after liver resection. Stripping film Kodak AR 10, exposure time 21 days, H & E staining. ×100.

Subtotal synchrony of DNA synthesis observed in the liver after this mode of a continuous infusion of HU has not been obtained before by any other method in a cell system in vivo. This model of a highly synchronized, differentiated normal parenchymal cell population might be a useful tool for biochemical investigations in vivo of events essential for G_1-S transition. Furthermore, the synchrony of DNA synthesis provides an excellent opportunity for studies on interactions between carcinogens and replicating DNA in vivo (manuscript in preparation).

Zusammenfassung. Intravenöse Dauerinfusion von Hydroxyharnstoff bis zu 40 h nach partieller Hepatektomie inhibiert den Beginn der DNA-Synthese in der Rattenleber. Nach Aufhebung des Blocks treten Hepatozyten subtotal synchronisiert in die DNA-Synthese ein. Ein ähnlich hoher Synchronie-Grad wurde bisher in vivo nicht erreicht.

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10 May 1974.

[22] Supported by grants from Deutsche Forschungsgemeinschaft.

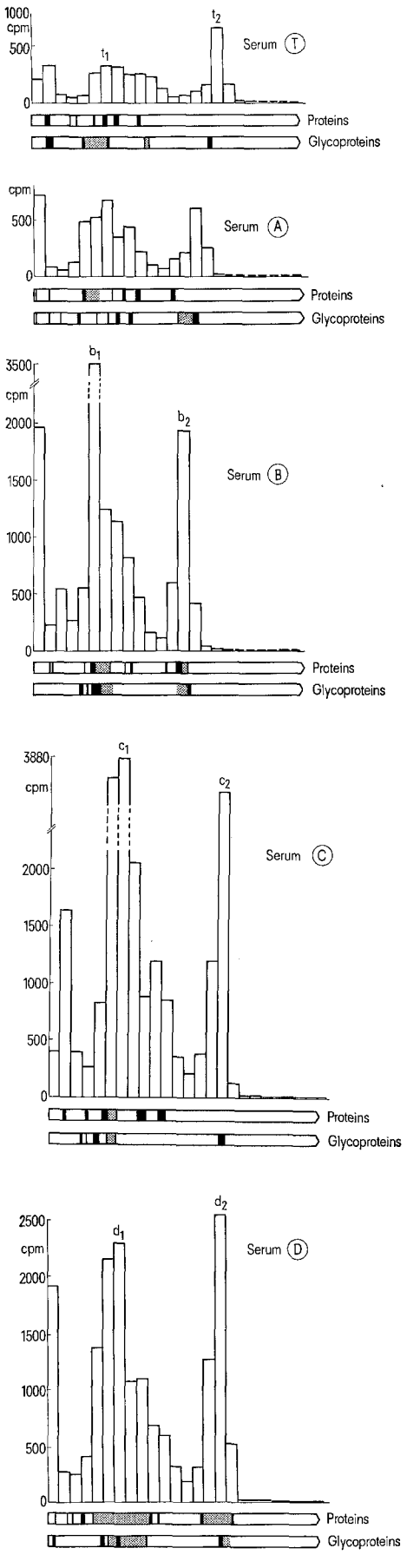
Influence of the Anti-Typhoparatyphoid Vaccines on the in vivo Activity of the Glucosaminyl-Transferases of Animals Infected by Myxovirus

Preceding work has shown that the infection of mice by myxovirus influenza leads to the hyperactivity of splenic and hepatic microsomal glycosyl-transferases, in vivo or in vitro, in acellular system. Under these conditions it seemed worthwhile to seek the in vivo influence of antibacterial and antiviral vaccinations, whether followed or not by viral infection, on these glycosylation activities of the proteins. The results previously obtained with the antipoliomyelitic vaccine have shown the important modifications at the level of the serum and of the soluble cytoplasmic stage of the infected animals hepatocytes[1,2]. The aim of this work is to show the influence on the rate of the glycosylations, of an anti-bacterial vaccine administered alone or before the influenza infection.

[1] C. Froger, A. Defrène and P. Louisot, Communication at the 10th Journées Biochimiques Latines, Rapallo 1971, No. 216.
[2] A. Defrène and P. Louisot, C. r. Acad. Sci., Paris *274*, D, 1853 (1972).

Table I. Experimental conditions of vaccination and viral infection for the 5 lots of animals T, A, B, C, D

Lots of animals	Programs
T	^{14}C-glucosamine ← 4 h → decapitation
A	TAB ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation
B	TAB ← 48 h → influenza ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation
C	TAB ← 96 h → influenza ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation
D	influenza ← 48 h → ^{14}C-glucosamine ← 4 h → decapitation



The animals used are mice from strain OF 1 (Iffa-Credo, France) with an average weight of 20 g. They are infected by the equine influenza virus adapted to mice by 18 successive runs through mice followed by 6 runs through strain OF 1. It is inoculated by intranasal means, under a volume of 0.1 ml, under light anaesthesia. The vaccine used is the TAB vaccine from the Institut Pasteur administered by deep s.c. means (0.2 ml per mouse at requisite dilution). The glycosylation of glycoproteins is followed by i.p. injection of 1-^{14}C-D-glucosamine (CEA, France, specific activity 51 Ci/*M*). Table I summaries the experimental conditions.

After decapitation of the animals, the serums, livers and spleens are removed. These organs are put in suspension in a *Tris*-HCl 0.05 *M*, sucrose 0.25 *M*, pH 7.5 (3 ml/g) buffer. The suspension thus obtained is homogenized by 10 strikes in the Potter-Elvehjem at +4 °C. The nuclei are eliminated in 10 min at 1000 *g*. The mitochondria are separated in 15 min at 10,000 *g*. The microsomes are sedimented out in 60 min at 100,000 *g*. After being replaced in suspension in a final deoxycholate at 0.4/100 ml, the ribosomes are separated from the endoplasmic membranes in 120 min at 200,000 *g*.

The serums, the soluble cytoplasmic phases and the membrane fractions are brought to a suitable protein concentration by dilution or concentration on a Schleicher and Schull ultrafilter, according to the case. The determination of the protein rates and the measurement of radioactivity is achieved under previously described conditions[3–5]. The polyacrylamide gel electrophoresis have been achieved in an Acrylophor-Pleuger apparatus.

Table II summaries the specific activities the non-dialysable 1-^{14}C-D-glucosamine of each sub-cellular fraction studied in spleen and liver. The electrophoretic separations of glycoproteins of the serum are diagrammized in Figures T, A, B, C, D.

The sum of these observations brings to light the following facts: 1. At the level of the serums, the administration of TAB vaccine alone leads to an increase of the specific radioactivity of the glycoproteins marked by the ^{14}C-glucosamine. On the electrophoretic profile of this serum (A), the specific radioactivity increase includes all the fractions; however a clearly marked band a_2 has not a corresponding band t_2 in the serum (T).

2. The administration of the influenza virus leads to a hyperactivity in the incorporation of the radioactive precursor (lot (D)); this hyperactivity appears in 2 principal zones in the electrophoresis, the peaks d_1 and d_2, and the homologuous peaks t_1 and t_2 of the control serums.

3. The administration of the TAB vaccine very clearly inhibits the post-influenza hyper-glycosylation reaction, and more clearly, the TAB vaccine has been more prematurely administered before the injection (inhibition more marked for lot (C) than (B)).

[3] A. DEFRÈNE and P. LOUISOT, Ann. Inst. Pasteur *122*, 529 (1972).
[4] A. DEFRÈNE and P. LOUISOT, Int. J. Biochem. *4*, 249 (1973).
[5] P. LOUISOT, C. FROGER and A. DEFRÈNE, Exposés a. Biochim. méd. *32*, 149 (1973).

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Electrophoretic separation in polyacrylamide gel (10 cm, 7mA); coloration of the proteins by Coomassie blue and of glycoproteins by periodate-fuchsine; counting of radioactivity in scintillating liquid after dissolution in oxygenated water.

4. Separately, TAB vaccination or infection by influenza virus leads to marked hyperglycosylation reactions. On the other hand, if the reactions follow each other in time, instead of summating, hyperglycosylation reaction cancels global reaction is very inferior to isolated reactions.

5. At the electrophoretic level we can consider that the peaks t, a, b, c and d correspond to homologous fractions but to different specific activities. It is not the same case for peaks t_2, b_2, c_2, especially the last two, whose migration speeds are slightly different.

Table II. Specific activities, expressed as cpm/mg of undialyzable subcellular protein fractions for lots T, A, B, C, D after 1-^{14}C-D-glucosamine injection

Lots / Fractions		T	A	B	C	D
Serum		3500	7288	6768	2118	15,200
Liver	Mb	3000	5500	5830	4860	8800
	Cell sap	1500	1970	3900	1740	3400
Spleen	Mb	255	955	210	750	810
	Cell sap	825	1370	1710	1310	1790

Mb = membrane

6. The effect of the TAB vaccine, alone or pre-influenza, more easily analyzed at the level of serum, leads to similar results in the liver or spleen subcellular fractions (Table II).

The study of in vivo incorporation of 1-^{14}C-D-glucosamine and the electrophoretic analysis of serum glycoproteins show that the preliminary injection of an antibacterial vaccine (TAB) very significantly disturbs the hyperglycosylation reaction which normally follows an influenza virus infection[6,7].

Résumé. Les résultats de l'incorporation in vivo de 1-^{14}C-D-glucosamine dans le foie et la rate, et l'électrophorèse en gel de polyacrylamide des glycoprotéines sériques, montrent que le vaccin TAB, injecté avant une infection à Myxovirus, perturbe qualitativement et quantitativement la réaction d'hyperglycosylation post-grippale.

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[6] A. DEFRÈNE and P. LOUISOT, C. r. Acad. Sci., Paris *276*, D, 1787 (1973).
[7] M. J. PÉSCHARD and P. LOUISOT, C. r. Acad. Sci., Paris, *278 D*, 1637 (1974).

Effect of Allyl Propyl Disulphide Isolated from Onion (*Allium cepa* L.) on Glucose Tolerance of Alloxan Diabetic Rabbits

The antidiabetic property of onion (*Allium cepa*) was first reported by COLLIP[1]. A depancreatectomized dog was kept alive for 66 days by 3 injections of onion extract during that period. Its blood sugar was controlled and the glycosuria disappeared. Since then many workers[2–8] have found that the hypoglycaemic principle in onion is also effective when given orally and that it can be steam distilled and extracted with ether solvents. Very recently another therapeutic use of onion, viz. fibrinolytic activity has also been reported by GUPTA et al.[9] and MENON et al.[10–12]. Even fried onion has been reported to be effective in lowering the blood glucose levels in clinical diabetes[13,14]. Considering the wide use of this vegetable, a study of the effects of its pure hypoglycaemic principle on experimental diabetes was felt worthwhile in order to elucidate its antidiabetic action. A preliminary study has shown that the volatile hypoglycaemic principle or any other active fraction of onion[5–7] on a single dose is not as effective as tolbutamide in lowering the fasting blood sugar of normal or alloxan diabetic rabbits. This, in addition to the findings of COLLIP, suggests that the principle in onion may be used for a few days to obtain any beneficial result. The present paper deals with the effect of the volatile hypoglycaemic principle, allyl propyl disulphide (APDS), isolated from onion on alloxan diabetic rabbits on a short term therapy.

Methods. Allyl propyl disulphide, C_3H_5–S–S– C_3H_7 was isolated from onion by the distillation procedure of PLATENIUS[15]. The active principle was isolated from the steam distillate by repeated extraction with ethylether in presence of 10% (W/V) sodium chloride. The ether extract was evaporated under reduced pressure and the oil left behind was used in this study (50 mg/100 g). The glucose tolerance test of the 12 rabbits made moderately diabetic with alloxan according to a previous procedure[6] was carried out and they were divided into 2 equal groups. Their body weights were noted (average 1.55 kg). 1 group was kept as control and the other group received APDS (dose 100 mg/kg/day) orally for a period of 15 days and at the end of this period glucose tolerance of both the groups were determined. During the period of experiment all the animals had the same rabbit feed.

[1] J. B. COLLIP, J. biol. Chem. *56*, 513 (1923); *57*, 65 (1923); *58*, 163 (1923).
[2] M. M. JANOT and J. LAURIN, C. r. Acad. Sci., Paris *191*, 1098 (1930).
[3] J. LAURIN, C. r. Acad. Sci., Paris *192*, 1289 (1931).
[4] H. KREITMAIR, E. Mercks Jber. *50*, 102 (1936).
[5] H. D. BRAHMACHARI and K. T. AUGUSTI, J. Pharm. Pharmac. *13*, 128 (1961).
[6] H. D. BRAHMACHARI and K. T. AUGUSTI, J. Pharm. Pharmac. *14*, 617 (1962).
[7] K. T. AUGUSTI, Indian J. med. Res. *61*, 1066 (1973).
[8] R. C. JAIN, C. R. VYAS and O. P. MAHATMA, Lancet *2*, 1491 (1973).
[9] N. N. GUPTA, R. M. L. MEHROTRA and A. R. SIRCAR, Indian J. med. Res. *54*, 48 (1966).
[10] I. S. MENON, R. Y. KENDAL, H. A. DEWAR and D. J. NEWEL, Br. med. J. *3*, 351 (1968).
[11] I. S. MENON, Br. med. J. *1*, 845 (1969).
[12] I. S. MENON, Br. med. J. *2*, 421 (1970).
[13] R. C. JAIN and K. N. SACHDEV, Curr. med. Pract. *15*, 274 (1971). – Lancet *2*, 1491 (1973).
[14] R. C. JAIN and K. N. SACHDEV, J. med. Prof. India *27*, 7991 (1971). – Lancet *2*, 1491 (1973).
[15] H. PLATENIUS, J. Agric. Res. *51*, 847 (1935).