the electrostatic charge or the hydrophobicity of the compound DNP may be such that it fits to a number of antibodies which are not related to DNP. Indeed PARKER and OSTERLAND[14] have shown that there is a hydrophobic region on the Fab fragment of a variety of immunoglobulins, which is capable of reacting non-specifically with benzenoid ligands. Confirming these reports, we observed weak interactions, when IgG isolated from a normal human serum pool was reacted with DNP-protein-conjugates by our technique. On the other hand, we could show that besides this unspecific binding the reaction of antibodies to DNA with DNP-protein-conjugates is much stronger.

Among the nucleoside- and nucleotide-protein-conjugates, adenosine-monophosphate-BSA and to a much lesser extent guanosine-, cytidine- and uridinemonophosphate-BSA reacted with the anti-DNA antibodies. It may not be excluded, however, that the weak interactions observed with the last three compound might be due to unspecific quenching, which occasionally occurs when an inhibitor is added at high concentrations.

The results reported might be of some relevance to the relatively frequent findings of myeloma proteins precipitating DNP-protein-conjugates and crossreacting with DNA[1–3], as they add further evidence to the speculations that the specificity of these myeloma proteins might be directed in fact against DNA or degradation products of DNA, instead of DNP.

Zusammenfassung. Kreuzreaktionen mit Dinitrophenyl-, Pyrimidin-, Purin- sowie Nucleosid- und Nucleotid-Protein-Konjugaten konnten bei Antikörpern gegen DNS, wie sie im Serum von Patienten mit Lupus erythematodes auftreten, nachgewiesen werden. Diese Resultate sind möglicherweise von Bedeutung für die Interpretation der auffällig hohen Frequenz von Paraproteinen, welche mit Dinitrophenylverbindungen reagieren.

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[14] G. W. PARKER and C. K. OSTERLAND, Biochemistry *9*, 1074 (1970).

[15] Acknowledgments. The authors wish to thank Miss E. ISCHI for her excellent technical assistance, Dr. H. W. BAENKLER (Erlangen) and Prof. P. H. LAMBERT (Geneva) for sera of patients with SLE.

[16] This investigation was supportec by the Swiss National Foundation for Scientific Research.

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Immunological Reactivity During Pregnancy in the Mouse

It is generally accepted that the placental filter is not an absolute barrier for foetal antigens, which can frequently stimulate the lymphatic system of the mother. This challenge results in the production of leucoagglutinins[1] and anti-HL-A antibodies[2] directed against foetal leucocytes antigens. In contrast, cell-mediated immune reactions against foetal antigens seem to be depressed in the mother. Recent interpretations of such a discrepancy are based on the depression or even abrogation of cell-mediated immune reactions by blocking antibodies, capable of coating the transplantation antigens of foetal cells[3] and/or those of maternal lymphocytes[2]. Alternatively the depression of cell-mediated immunity could be due to the particular hormonal balance during pregnancy[4]. These 2 different interpretations however do not exclude each other, because the proposed subtle interplay between humoral and cellular immunity during pregnancy[2] could hypothetically be favoured or even induced by some hormones secreted in that condition. In order to decide between these possibilities cell mediated immune reactions (contact allergy) and antibody response (PFCs capacity) have been quantitatively evaluated at different periods of pregnancy in the mouse.

Materials and methods. Inbred C3H and outbred Swiss albino young adult mice have been used. Virgin females of both strains have been mated with syngeneic or allogeneic males for a 24 h period. In order to get identical experimental conditions in pregnant and in virgin females, time of sensitization, matings and tests were clearly defined. Contact allergy has been induced[5] by painting the shaved abdomen of mice with picryl chloride (BDH, Chemicals, Poole, England) dissolved in absolute ethanol; the reaction has been quantitatively evaluated by measuring with a Panter micrometer the ear thickness before, at 24 and 48 h after a second application with the sensitizing agent dissolved in pure olive oil. Animals were either sensitized before pregnancy and then tested at different stages of pregnancy or sensitized at 10 days of pregnancy and tested 20 days after delivery.

Antibody response has been evaluated by measuring the number of plaques-forming cells (PFCs) against sheep erythrocytes (SRBC) according to the method of JERNE et al.[6] Animals were immunized by an i.p. injection with 0.1 ml of a 20% suspension of SRBC in physiological saline and sacrificed 4 days after immunization.

Results. C3H female mice sensitized to picryl chloride before pregnancy and then tested during pregnancy show a clear depression of the allergic reactions both at 24 and 48 h after challenge (Table). The group tested 30 days after delivery shows a complete normalization of the reaction. On the other hand C3H females sensitized the 10th day of pregnancy and tested 20 days after delivery react in a normal way (Table). In contrast to these findings on cellular immunity, the antibody response increased during pregnancy both in C3H and in Swiss Albino mice (Figure). The increments are statistically significant only in the groups sacrificed at 7, 11 and 14 days of gestation. In the groups sacrificed at 17, 19 and 21 days after conception (the last group was tested 1 day after delivery), the number of PFCs per spleen drops to the level present in non pregnant females. We have observed a 5–10 fold increase in pregnant C3H mice at 14 days of

[1] R. PAYNE and M. R. J. ROLFS, Clin. Invest. *37*, 1756 (1958).

[2] R. CEPPELLINI, *Progress in Immunology* (Academic Press, N.Y. 1971), p. 1573.

[3] K. E. HELLSTRÖM, I. HELLSTRÖM and J. BRAUN, Nature Lond. *224*, 914 (1969).

[4] S. KASAKURU, Proc. 6th Leucocyte Culture Conference (Ed. M. R. SCHWARZ; Acad. Press, N. Y. 1972), p. 711.

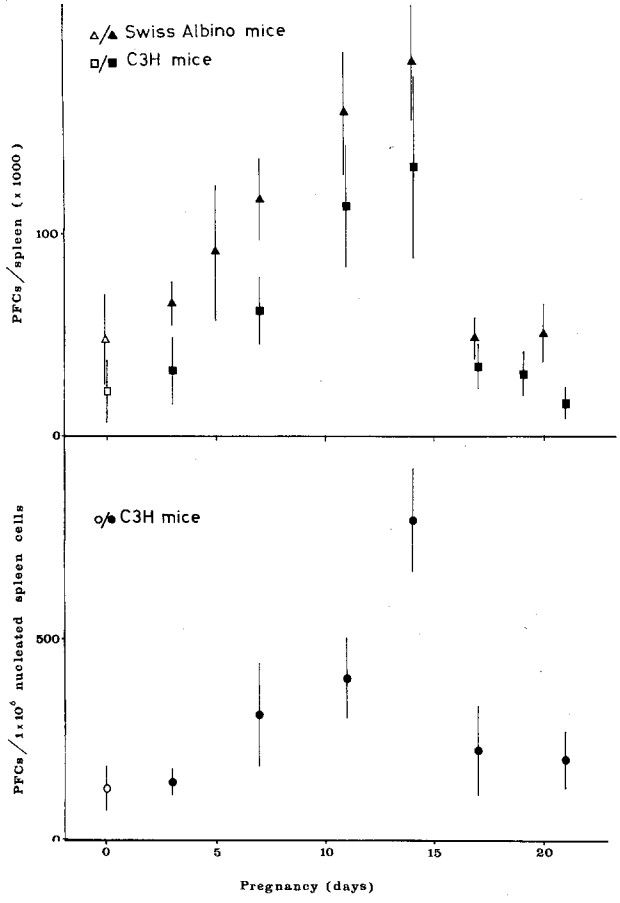
[5] G. L. ASHERSON and N. PTAK, Immunology *15*, 405 (1968).

[6] N. K. JERNE, A. A. NORDIN and C. HENRY, in *Cell-Bound antibodies*, Wistar Institute Symposium Monograph No. 3, Ed. V. DEFENDI; Wistar Institute Press, Philadelphia 1963), p. 109.

Contact allergy to picryl chloride in pregnant or non-pregant C3H mice

Experimental groups		Days after conception at test	Days between sensitization and test	Test agent	Increase of ear thickness (10^{-3} cm) at 24 h	at 48 h
Non-pregnant	(8)[a]	0	60	Picryl chloride	5.75 ± 1.93	10.5 ± 2.03
				Oil (control)	0.35 ± 0.72	0.72 ± 0.69
Pregnant	(6)	10	60	Picryl chloride	2.55 ± 1.93	3.07 ± 2.20
				Oil (control)	0.62 ± 0.37	0.88 ± 1.12
Pregnant	(5)	15	60	Picryl chloride	3.10 ± 2.37	4.15 ± 0.73
				Oil (control)	0.35 ± 1.05	0.26 ± 1.25
Pregnant	(2)	21 (delivery)	60	Picryl chloride	1.55	4.12
				Oil (control)	0.37	0.78
Pregnant	(7)	51	60	Picryl chloride	7.25 ± 2.07	10.04 ± 2.73
				Oil (control)	0.38 ± 0.92	1.24 ± 0.75
Pregnant	(5)	40	30	Picryl chloride	7.36 ± 2.12	11.05 ± 1.58
				Oil (control)	0.75 ± 0.37	1.52 ± 0.53
Non-pregnant	(4)	0	30	Picryl chloride	6.85 ± 2.36	10.51 ± 2.96
				Oil (control)	0.81 ± 2.28	1.12 ± 0.62

[a] Number of animals in brackets.



PFCs capacity in pregnant mice at different stages of gestation. Animals have been injected i.p., 4 days before sacrifice, with 0.1 m of a 20% sheep erytrocytes suspension in saline. PFCs were evaluated according to the method of JERNE et al.[6]. Closed symbols indicate pregnant mice; open symbols non pregnant controls. △/▲, PFCs/spleen in Swiss Albino mice; □/■, PFCs/spleen in C3H mice; ○/●, PFCs/1 × 10^6 nucleated spleen cells in C3H mice; I, standard errors.

pregnancy when compared to virgin females, while a 3–5 fold increase was noted under similar conditions in Swiss Albino mice (Figure). The increment of the number of PFCs per spleen during pregnancy is not simply due to an aspecific increase of all kinds of nucleated cells in the spleen because also the number of PFCs per million spleen cells is higher in pregnant than in virgin females (Figure).

Discussion. The present experiments show that contact allergic reactions to picryl chloride in C3H female mice, are depressed during pregnancy. These results are in agreement with the impairment of cell-mediated immunity, which has been observed in animals[4] or in humans[2,7]. Such a depression does not seem to be specifically limited to the reaction against transplantation antigens of the new born but it involves the reactions against unrelated individuals[2], intracellular microorganisms[8,9] and, as in our case, against chemical sensitizing agents. It is of interest to note that, at least in our experimental model, the process of sensitization is not appreciably affected by the state of pregnancy whereas the actual expression of the allergic reaction is disturbed. In fact, female mice sensitized to picryl chloride during pregnancy and tested 20 days after delivery show reactions comparable to those observed in virgin females.

In contrast to contact allergic reactions humoral immune responses against SRBC, as measured by plaques formation, are significantly increased during pregnancy. These findings do not seem to be a casual observation because increased plaque formation has been found[10] in old retired female breeders when compared to virgin females of the same age, suggesting that repeated pregnancies could affect the number of PFCs precursors.

[7] D. T. PURTILO, H. M. HALLGREN and E. J. YUNIS, Lancet *1*, 769 (1972).
[8] I. A. D'CRUZ, S. G. BALANI and L. S. IYER, Obstet. Gynec., N. Y. *31*, 440 (1968).
[9] R. E. PICKARD, Am. J. Obstet. Gynec. *101*, 504 (1968).
[10] G. J. V. NOSSAL, A. E. BUSSARD, H. LEWIS and J. C. MAZIE, in *Developmental Aspects of Antibody Formation and Structure* (Ed. J. STERZL and I. RIHA; Czechoslovak Acad. Press, Praha 1970), p. 655.

From these findings it seems, therefore, that some physiological conditions present during pregnancy can influence the proliferation or actual performance of antibody forming cells and of cells involved in contact allergy and possibly in cell-mediated immunity. The resulting modifications of the humoral and cellular immune response can be reasonably explained if we assume the existence of an extralymphatic regulatory mechanism exerting its action on the immune reactivity during pregnancy[4]. Such a control mechanism could be represented by some hormones, which are synthetized in higher amounts during pregnancy, and which are known to exert a depressive action on cell-mediated immunity[11–13]. It is difficult to decide at the present time whether one or more of the pregnancy hormones can be involved in such a situation, and, particularly, in our experimental model. It can be postulated, however, that they should be able to stimulate the humoral immune response, as it has been reported in other experimental models[14], while depressing directly or through the increased production of blocking antibodies cell-mediated immune reactions against the foetus.

Riassunto. Le reazioni allergiche da contatto indotte dal cloridrato di picrile e le risposte anticorpali agli eritrociti di montone sono state quantitativamente valutate in topi femmine nel corso della gravidanza. Mentre le reazioni allergiche sono diminuite nelle femmine gravide rispetto alle vergini, le risposte anticorpali risultano significativamente aumentate. Tali dati fanno supporre l'esistenza nel corso della gravidanza di un controllo ormonale delle risposte immunitarie che potrebbe influenzare positivamente le reazioni meterno-fetali.

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7 February 1973.

[11] M. D. KAYE and W. R. JONES, Am. J. Obstet. Gynec. *109*, 1029 (1971).
[12] S. R. WALTMAN, R. M. BURDE and J. BERRIOS, Transplantation *11*, 194 (1971).
[13] J. S. MUNROE, J. Reticuloendothelial Soc. *9*, 361 (1971).
[14] N. FABRIS, W. PIERPAOLI and E. SORKIN, Clin. expl. Immun. *9*, 227 (1971).

Phagocytosis and Nitroblue Tetrazolium Reduction in Uremia

Polymorphonuclear neutrophil (PMN) mobilization appears to be normal in uremic patients[1,2], but the integrity of further steps in engulfment and killing of bacteria has been questioned[3,4]. We have investigated PMN function in a series of uremic patients, as measured quantitatively by latex phagocytosis and by reduction of a colorless dye, nitroblue tetrazolium (NBT), to black cytoplasmic deposits within the cell. The presence of reduced NBT within the PMN indicates oxidative metabolism has occurred.

Ten hospitalized uremic patients were studied when they were free of infection. Eight had never been dialyzed;

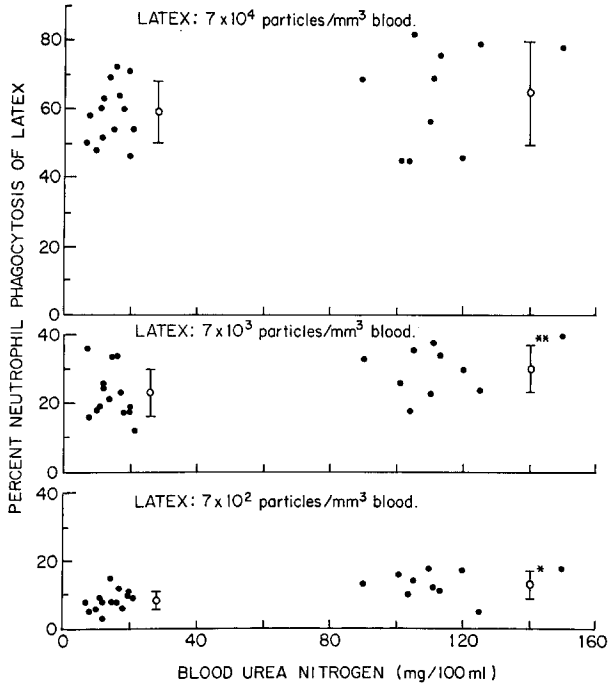

Fig. 1. Latex phagocytosis by neutrophils from uremic and control patients. At each of the 3 latex concentrations tested, brackets enclose the mean and standard deviation of particle uptake for controls on the left and uremic patients on the right. *, $p < 0.01$; **, $p < 0.05$.

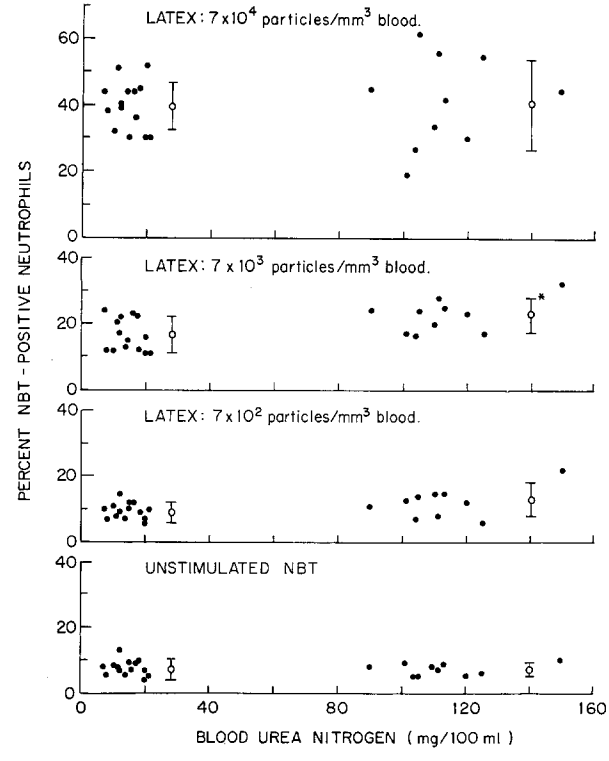

Fig. 2. NBT scores of neutrophils from uremic and control patients. Brackets enclose the mean and standard deviation of the NBT scores for controls on the left and uremic patients on the right. *, $p < 0.01$.