pregnancy were: Finding of albumin in the urine, increase in blood pressure above 150/100 mg Hg, swellings.

*Results.* Statistical evaluations did not reveal any association between the age of the women, or between the advance of pregnancy, on the one side and elevation of the antibody titre, or the incidence of toxemia on the other. This made it possible to treat each of the groups as a whole while comparing their immunological reactivity.

Fig. 1

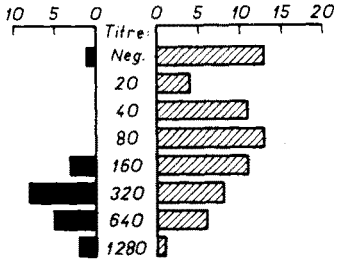



Fig. 2

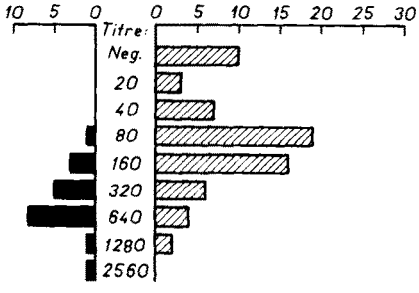
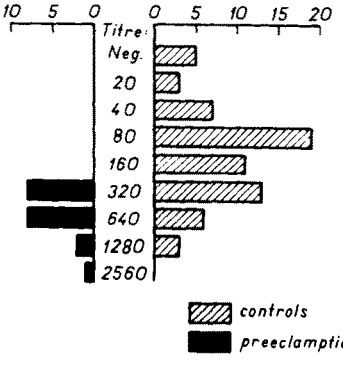



Fig. 3




It was found that already on the 7th day after the antigenic impulse the titres of incomplete antibody to Brucella antigen were statistically significantly higher in the preeclamptic group than in the control group ($P = 0{\cdot}05$). This difference still increased on the 14th and the 21st day and became higly significant ($P = 0{\cdot}01$). Figure 1 and 2 show the result of the titration of incomplete antibody on the 14th and the 21st days following immunization, and Figure 3 brings a comparison of all individual top titre levels irrespective of the time elapsed since immunization. All these graphs, but especially clearly the last one, bring to light the elevation of incomplete antibody titres in the group of preeclamptic women in comparison with the controls.

*Discussion.* The increase in the ability to produce incomplete antibody has already repeatedly been demonstrated for acute rheumatic fever[1,2], and acute glomerulonephritis[3]. The observations which are reported here suggest that preeclamptic conditions may also be classed with the diseases characterized by an increased immunological reactivity. On the other hand, our results do not reveal the mechanism by which hyperreactivity predisposes to pathological developments. It is true that in the group of preeclamptic women there was not a single case in which incomplete antibody to Brucella antigen was not present in a relatively high titre. Inversely, there were several pregnant women in whom signs of preeclampsia were lacking but who also reacted to the Brucella stimulus with a significant titre. Thus, it may well be that hyperreactivity is only one of the essential preconditions for the genesis of toxemia of pregnancy. Some further factor or complex of factors may be involved in this pathogenesis. For acute rheumatic fever and for acute glomerulonephritis, the etiological role of streptococcus pyogenes has been established: no similar agent has so far been detected for preeclampsia. Possible complicity of the foetus has been suggested by a number of workers but as yet not clearly defined. The auto-immunization processes described may well be conditioned primarily by hyperreactivity, but one should not leave out of account further possible factors, such as the easiness with which the antigenic structure of tissues may change, a certain antigenic affinity of different organs, as well as external influences which may also come into play in antigenic changes in the organism.

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*Résumé*

Après une seule injection d'un vaccin brucellique on trouve chez les femmes atteintes d'éclampsie une production d'anticorps incomplets significativement plus élevée que chez les femmes dont la grossesse a été normale.

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## Maintenance of the Ability of Cells Cultivated *in vitro* to Commence Formation of Antibodies

It has been demonstrated in a number of papers[1] that mesenchymal cells removed from immunized donors continue to produce antibodies in tissue cultures. It is, however, still doubtful whether production of antibodies can be demonstrated in tissue cultures if the tissue is explanted shortly after injection of the antigen, i.e. up to 48 h, during the inductive phase. The majority of experiments in which antigen was added to an explantate of tissue from a non-immunized donor gave negative results[2]. Recently a few positive results have been reported[3].

In experiments performed during recent years, it was shown in our laboratory[4] that spleen tissue explanted

[1] A. FAGRAEUS, Acta med. scand. *130*, Suppl. 204 (1948). – G. J. THORBECKE and F. J. KEUNING, J. Immunol. *70*, 129 (1953); J. infect. Dis. *98*, 157 (1956). – A. B. STAVITSKY, J. Immunol. *75*, 214 (1955). – D. STEINER and H. ANKER, Proc. nat. Acad. Sci., Wash. *42*, 580 (1956).

[2] R. C. PARKER, Science *85*, 292 (1937). – A. J. SALLE and W. A. MCOMIE, J. Immunol. *32*, 157 (1937). – D. STEINER and H. ANKER, Proc. nat. Acad. Sci., Wash. *42*, 580 (1956).

[3] W. HÖPKEN, Virchows Arch. *325*, 39 (1954). – K. M. STEVENS and J. M. MCKENNA, Nature *179*, 870 (1957); J. exp. Med. *107*, 537 (1958).

[4] J. ŠTERZL and M. RYCHLÍKOVÁ, Czechoslov. Microbiol. *2*, 334 (1957); Folia biol. *4*, 11 (1958).

shortly after immunization and cultivated in both a natural medium (Tyrode solution and equal part of rabbit serum) and in a synthetic medium (Parker's medium 199 + 0·5% homologous, i.e. rabbit albumin) does not form antibodies. They could also not be demonstrated in a synthetic medium that was further concentrated by pressure dialysis and lyophilization. Cultivation of tissues explanted 24 h after immunization always gave negative results. Explantation of tissues 48 h after immunization gave a positive result only in one out of 11 cases and only after concentration of the cultivation medium. In control experiments in which spleen tissue was explanted and cultivated when antibody formation was fully developed, e.g. after several doses of antigen, antibodies could be demonstrated directly in the medium and the antibody titre increase was proportional to the concentration. Antibodies could never be shown to be present in experiments in which normal explanted tissue was mixed with antigen *Brucella suis in vitro* and cultivated for up to 9 days[5]; not even if the tissue was first stimulated by the *Salmonella paratyphi* B antigen or purified lipopolysaccharide as was done by STEVENS.

Cultivation experiments clearly showed that it is not possible to realise the first inductive phase of antibody formation in tissue cultures. Using only the tissue culture method, it was not possible to decide whether tissue cultures do not produce antibodies because the cellular mechanism of antibody formation is inactivated – as was shown by HARRIS using incubation of cells at 37°C[6] – or whether the inductive phase is more sensitive to unfavourable physical and metabolic conditions in tissue cultures – as to the effect of X-rays[7] and cortisone[8] – or whether cells capable of reacting with the antigen cannot divide mitotically in tissue cultures and differentiate into cells producing antibodies.

We attempted to solve this problem by combining cultivation of spleen cells in tissue cultures with cultivation of cells *in vivo*, i.e. with cell transfer into the peritoneum of newborn rabbits. Infant animals and embryos, although not themselves capable of reacting to an antigen by antibody formation, provide cells with a favourable environment in which they can multiply, differentiate, and commence antibody formation[9].

The experiment was arranged as shown in Figure 1. Isolated spleen cells are mixed with corpuscular or liquid antigen of *Brucella suis*. Part of the cells at a concentration of $60 \times 10^6$/ml is transferred into the peritoneum of infant rabbits. Antibody formation by the cells was studied in the serum of the infant animals by agglutination[9]. The remaining part of the cells is transferred to a tissue culture in an Evans apparatus[10] or an Erlenmayer flask (vol. 250 ml, 10 ml cultivation medium). Spleen cells were added to Parker's medium 199[11] + 0·5% rabbit albumin

[5] J. ŠTERZL, Abstracts VII[th] Int. Congr. Microbiol., p. 226 (1958).

[6] S. HARRIS, T. N. HARRIS, and M. B. FARBER, J. Immunol. *72*, 148 (1954).

[7] C. G. CRADDOCK and J. S. LAWRENCE, J. Immunol. *60*, 241 (1948). – H. I. KOHN, J. Immunol. *66*, 525 (1951). – F. J. DIXON, D. W. TALMAGE, and P. H. MAURER, J. Immunol. *68*, 693 (1952). – W. H. TALIAFERRO, L. G. TALIAFERRO, and E. T. JANSSEN, J. infect. Dis. *91*, 105 (1952).

[8] K. BERGLUND and A. FAGRAEUS, Atti VI. Congr. int. microb. *2*, 231 (1953).

[9] J. ŠTERZL, Folia biol. *1*, 193 (1955); *3*, 1 (1957). – J. ŠTERZL and Z. TRNKA, J. hyg. epid. Microbiol. *1*, 292 (1957). – Z. TRNKA, Nature *181*, 55 (1958). – M. HOLUB, Nature *181*, 122 (1958).

[10] V. J. EVANS, W. R. EARLE *et al.*, J. nat. Cancer Inst. *11*, 907 (1951). – D. S. DANES, Exp. Cell Res. *12*, 169 (1957).

[11] J. F. MORGAN, H. J. MORTON, and R. C. PARKER, Proc. Soc. exp. Biol. Med., N. Y. *73*, 1 (1950).

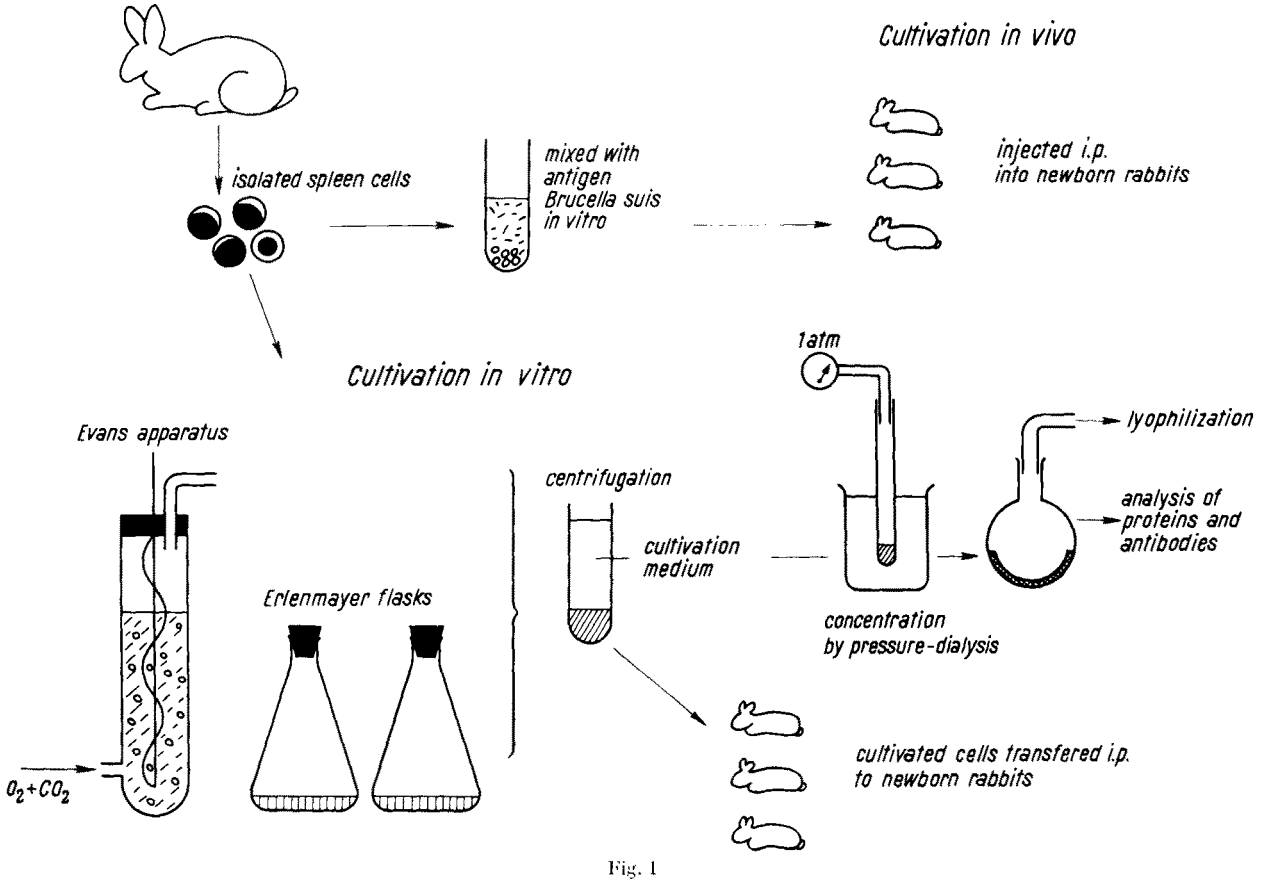


Fig. 1

so that the final cell concentration was $5 \times 10^6$/ml medium. They were aerated with 96% $O_2$ + 4% $CO_2$ and cultivated at 37°C for 6, 12, 24, 48 h up to 9 days. At the same time, a control series was always started, prepared under the same conditions, and kept at 4°C; 200 ml of medium were used in each experiment. The number of lymphoid cells counted in a Bürcker chamber and the viability test with trypan blue were determined every day of cultivation. After terminating cultivation at the times indicated above, the medium was concentrated and analysed for antibodies using agglutination with *Brucella suis* antigen. $\gamma$-Globulin was determined electrophoretically. After centrifugation, the cultivated cells were adjusted to the same concentration as for transfer to infant rabbits, i.e. to $60 \times 10^6$ cells/ml. This suspension of cultivated cells was again transferred to newborn rabbits.

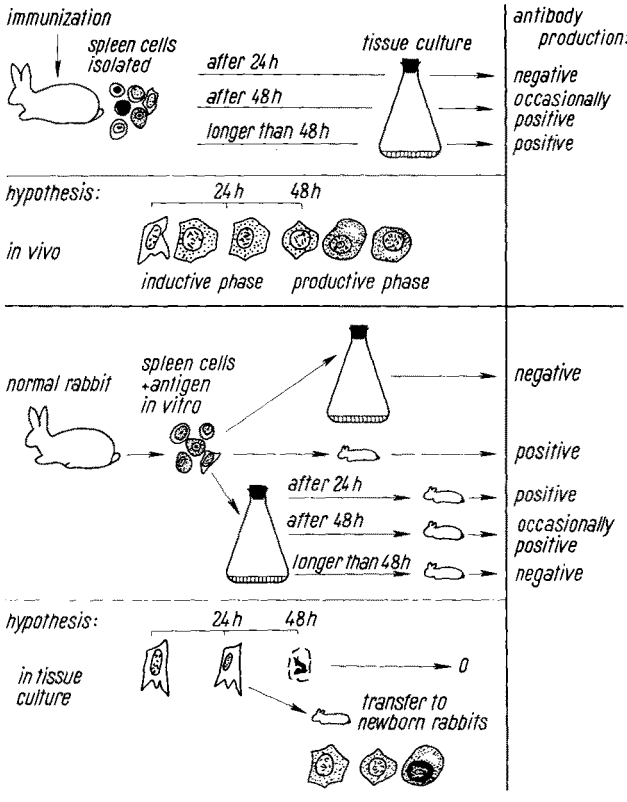


Fig. 2

Antibodies were never found in any experiment with cultivation of cells together with the antigen *in vitro*, not even after concentrating the culture medium 40 times. If, however, the cultivated cells were transferred after 6, 12, 24, and occasionally also 48 h cultivation to infant rabbits, antibody formation was induced (titre 1:32–256). If cells were transferred after longer cultivation (72 h to 9 days) formation of antibodies could never be demonstrated after transfer to infant rabbits.

Figure 2 summarizes the results of cultivation and the conclusions of the experiment. If spleen cells were taken from an immunized donor, antibodies could only be demonstrated if cells already producing antibodies were used for tissue culture. If isolated cells from a non-immunized donor were cultivated with an antigen *in vitro*, antibody formation in the tissue culture could never be demonstrated under any of the experimental conditions. Mixing the same cells *in vitro* with the antigen and subsequently transferring them to infant rabbits always resulted in antibody formation. If these cells were cultivated and transferred after certain time intervals into infant rabbits it was demonstrated that the ability to form antibodies in a tissue culture is maintained for only 24–48 h after explantation; then it disappears.

The results are interpreted to indicate that contact of the antigen with primitive mesenchymal cells[12] and their further division and differentiation[13] are necessary for antibody formation to commence – for the inductive phase. As long as the formation of productive cells is not terminated under conditions permitting these changes, i.e. *in vivo*, formation of antibodies in tissue cultures does not occur. Cells able to commence antibody formation do not lose this ability *in vitro* and survive there for a certain period. Formation of antibodies is only realised if they are transferred to suitable conditions, i.e. when cultivated *in vivo*.

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*Division of Immunology, Institute of Biology, Czechoslovac Academy of Science, Praha, September 20, 1958.*

*Zusammenfassung*

Aus einem normalen, nicht immunisierten Kaninchen isolierte Milzzellen, welche *in vitro* 6, 12, 24 und 48 h kultiviert und nachher zusammen mit *Brucella-suis*-Antigen in das Peritoneum neugeborener Kaninchen übertragen wurden, erzeugen Antikörper. Die Antikörper werden im Serum der Empfänger, das heisst neugeborener Kaninchen, welche selbst noch nicht zur Antikörperbildung befähigt sind, festgestellt. Falls die Milzzellen nach 72stündiger oder längerer Kultivierung auf neugeborene Kaninchen übertragen werden, ist diese Übertragung niemals von Antikörperbildung begleitet.

[12] J. ŠTERZL, *Mesenchymal tissue during immunisation and infection* (Praha 1954).

[13] R. W. WISSLER, F. W. FITCH, M. F. LAVIA, and C. A. GUNDERSON, J. cell. comp. Physiol. *50*, Suppl. 1, 265 (1957).

## Meccanismo dell'azione attivatrice esplicata dal Guanosintrifosfato (G.T.P.) sui sistemi enzimatici ossidasici dei grassi. Nota IV

I sistemi enzimatici ossidasici dei grassi preparati dal fegato di cavia sono attivati dall'A.T.P., dal G. T. P. e dall'α-chetoglutarato[1-3]. Il G. T. P. e l'α-chetoglutarato sembrano agire con meccanismo analogo, diverso da quello esplicato dall'A. T. P. Poichè il processo di attivazione consiste nel fornire energia al sistema enzimatico ossidasico dei grassi per la sintesi degli acil-Coenzima A – sintesi indispensabile affinchè il processo ossidativo possa svolgersi – si può prospettare che tale energia possa essere fornita dal G. T. P. e dall'α-chetoglutarico con due meccanismi: 1) o indirettamente nel senso che sia il G. T. P. che l'α-chetoglutarato forniscono energia per la sintesi del succinil-CoA secondo le seguenti reazioni *a*) e *b*):

*a*) G. T. P. + acido succinico + CoA = succinil-CoA + G. D. P. + Pi;

*b*) α-chetoglutarato + CoA $\xrightarrow[+\,1/2\ O_2 - CO_2]{}$ succinil-CoA

e a sua volta il succinil-CoA potrebbe trasferire il CoA direttamente all'acido grasso secondo la seguente reazione *c*):

[1] M. SACCHETTO e C. R. ROSSI, Exper. *14*, 253 (1958).

[2] C. R. ROSSI e M. SACCHETTO, Exper. *14*, 254 (1958).

[3] M. SACCHETTO e C. R. ROSSI, Exper., in corso di pubblicazione.