Anti-N from *Moluccella laevis*

Anti-N agglutinins from the seeds of *Vicia graminea*[1], other *Viciae* and various *Bauhineae*[2,3] are known; of these, *Vicia graminea* anti-N is the most satisfactory. *Vicia graminea* is indigenous to South America; the seeds are therefore not readily available elsewhere.

We here report the finding of a good anti-N agglutinin along with anti-A in extracts of the seeds of *Moluccella laevis* (Bells of Ireland, Shell Flower, Molucca Balm) of the Natural Order, Labiatiae. The seeds are readily available from seed merchants in Britain and, we have no doubt, in other parts of Europe.

Moluccella laevis seeds, purchased from Thompson & Morgan Ltd., Ipswich, were extracted by the method of BOYD and REGUERA[4]. Fresh crude extracts were tested against freshly collected thrice-washed capillary specimens of human group B or O red cells suspended in isotonic saline solution (1 vol. of packed cells to 9 vol. of saline (10%) for tile tests, and to 24 vol. of saline (4%) for tube tests).

The agglutinin is relatively N-specific; appropriate dilution readily makes the extract N-specific. Specificity is quickly and clearly demonstrated either by avidity of agglutination on a tile or by conventional titration in tubes.

There are already indications that the *Moluccella* agglutinin makes a sharp quantitative estimate of N-antigen strength. Thus, the agglutinin promises to be of value as a research tool in the MN blood group system, in which 'dosing' reagents are of value in detecting variants of the M and N antigens.

As with the *Vicia graminea* anti-N, the action of anti-N on neuraminidase-treated NN or MN red cells is enhanced.

The *Moluccella laevis* agglutinin has been reported as non-specific by HOSSAINI[5]; this could be due to variation among strains of the plant or, more likely, to the fact that the tests were read after centrifugation.

Most specific seed agglutinins have been obtained from the genus *Leguminosae*. The *Moluccella laevis* agglutinin is the second specific agglutinin to be found in the genus *Labiatiae*, the first being the anti-A agglutinin of *Hyptis suaveolens*[6]. A wider search among seeds of this genus might be rewarding.

Further studies of the *Moluccella laevis* agglutinin are in progress and a complete report will appear elsewhere.

Zusammenfassung. In Samen von *Moluccella laevis* gelang der Nachweis von Anti-N-Agglutinin mit besonders starker Wirkung auf NN-Erythrozyten.

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Regional Blood Transfusion Service, Birmingham 15 (England), 11 June 1969

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A New Method of Examining the Stimulatory Effect of *Serratia marcescens* Endotoxin on Non-Specific Resistance to Infection

Owing to the wide range of their biological activities, endotoxins prepared from Gram negative bacteria have been at the centre of interest for the recent decade. Several authors have reported increased resistance of endotoxin-treated experimental animals to various bacterial[1,2], viral[3,4] and fungal[5,6] infections.

But it has supposed to be a non-specific resistance-increasing effect whose mechanism has not yet been unequivocally clarified. The present paper is a report of studies on the mechanism of action of the increase of non-specific resistance to infection by endotoxin pre-treatment.

Material and method. The experiments were performed on randomized male Wistar rats weighing 100–150 g. Trypanosoma equiperdum strain maintained in this laboratory in mouse passages since 1942 was used for infection. This protozoon develops exclusively in the blood and has no intracellular stage and, being very small, can be readily counted in a Buerker counting chamber.

Male rats, 100–150 g, were given i.v. fresh infected blood obtained by cardiac punction, with the initial count adjusted to 2×10^7/ml. In the blood of the infected animals trypanosome counts were determined at 1 h intervals. The blood samples taken from the tail vein were diluted 200-fold with low heparin containing saline and the actively moving organisms were counted in a Buerker chamber. The limit of error of this method was $\pm 5\%$.

The endotoxin used in the experiments was extracted from *Serratia marcescens* with Boivin and Mesrobeanu's method. 1 ml of the preparation contained active substance from 4×10^9 germs. Its MLD for the rat was 0.5 ml/100 g. The animals were treated with rising doses of endotoxin on 6 occasions at 48 h intervals. The last dose, which was twofold of the MLD, caused no symptoms, indicating the development of endotoxin tolerance in the meantime. Intravenous infection with trypanosomes was carried out 48 h after the last endotoxin dose.

Results and discussion. Prior to the experiments the characteristic properties of the *Trypanosoma* strain were determined. The growth curves in control rats indicated an immediate logarithmic replication of the trypanosomes; thus in the control group the animals died at a trypanosome count of $2–2.5 \times 10^9$/ml blood. From the growth curves plotted out from trypanosome counts in 26 rats, the duplication time of trypanosomes was assessed as 5.5 h and the tangens alpha of the logarithmic phase as 0.0547[7].

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Length of lag phase and duplication time of *Trypanosoma equiperdum* in control and endotoxin treated rats

Control						Endotoxin treated[a]		
Serial No.	Lag phase (h)	Duplication time (h)	Serial No.	Lag phase (h)	Duplication time (h)	Serial No.	Lag phase (h)	Duplication time (h)
1	0	5.6	14	0	5.4	1	11.1	4.7
2	0	6.0	15	0	5.9	2	20.8	5.2
3	0	5.2	16	0	5.7	3	17.1	6.1
4	0	5.4	17	0	6.0	4	13.9	5.5
5	0	6.0	18	0	4.7	5	9.7	5.1
6	0	5.6	19	0	4.9	6	17.8	5.8
7	0	5.1	20	0	5.3	7	15.1	5.6
8	0	5.5	21	0	5.3	8	16.8	5.3
9	0	4.9	22	0	5.6	9	12.5	5.7
10	0	6.0	23	0	5.4	10	13.5	5.3
11	0	5.4	24	0	5.9			
12	0	6.1	25	0	5.4			
13	0	5.5	26	0	5.9			
				Average:	5.5		Average: 14.8	5.4
				S.E.:	0.025		S.E.: 1.06	0.05

[a] Received a total of 6 doses of endotoxin at 2-day intervals.

Serial pretreatment with endotoxin resulted in a characteristic alteration of the trypanosome growth curves. Infection was generally followed by a 14.8 h lag phase, and the logarithmic phase started only afterwards.

The Table shows the lengths of lag phases and duplication times measured on endotoxin-treated and control rats. The duplication times measured in the logarithmic phase did not differ in the experimental and control group. This implies that in the logarithmic phase the growth rate of trypanosomes was the same in rats treated and not treated with endotoxin.

The lengths of the lag phases were graphically extrapolated to the abscisse by means of points plotted out during the logarithmic phase[8].

With trypanosome count of 2×10^7/ml, the counts read at 1 h intervals during the logarithmic phase fluctuated from 1.9×10^7 to 2.1×10^7 owing to the $\pm$ 5% error.

The experimental animals died, similarly to the controls, at a trypanosome count of 2–2.5 $\times 10^9$/ml blood, thus the delay caused by the 14.8 h lag phase was realized also in the time of exit.

It appears that the length of the lag phase is an appropriate marker of the endotoxin-induced increase of non-specific resistance. Investigations on this basis are scheduled to be carried further along 2 lines: as a method, the above procedure seems to be suitable for the assessment of the resistance-increasing effect of various preparations; on the other hand, though we are aware that the experiment described above is just an approximate model, we believe that the clarification of the mechanism of the appearance of lag phases will help to disclose the mechanism of action of endotoxin induced resistance.

Zusammenfassung. In mit Endotoxin chronisch behandelten Ratten verändert sich die Vermehrungskurve des *Trypanosoma equiperdum* charakteristisch.

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Studies on Lysosomes in Rat Heart Cell Cultures. II. The Effect of Exogenous Lysosomes[1]

Since DE DUVE's[2] discovery of the lysosomes, there has been a dispute about their function and significance in cell physiology and pathology[3–8]. FRIEDMAN et al.[9] were unable to show lysosomal or any other cellular alterations in beating rat heart cell cultures, using 10 µg/ml of vitamin A. When greater concentrations of the vitamin (100 µg/ml and 1000 µg/ml) were used, a cytopathic effect, which appeared to be due to a primary alteration of the cell membrane rather than to lysosomal damage, was obtained. The present study deals with the effect of exogenous lysosomes and the synergistic effect of chlorpromazine or dimethylsulphoxide and vitamin A on heart tissue culture.

Materials and methods. Beating rat heart cell cultures were prepared according to the technique described previously[9].

Preparation of lysosomal fraction. Rat, guinea-pig and mouse liver lysosomes were prepared according to the method described by WEISSMANN and THOMAS[10]. 2 ml of 20% of the large granule fraction in lactalbumin hydrolysate (LAH) medium[9], supplemented with 15% inactivated new-born calf serum, were added to each heart cell culture.

Rat liver lysosomal enzymes. The large granule fraction was ultrasonicated twice, for 20 sec, at 4 °C in a MSE ultrasonicator. The fluid was examined for lysosomes