2000 rpm for 10 min; the serum was then pipetted off and used for testing.

Hemagglutination tests were run as described by STAVITSKY[5] with the following modifications. Formalinized rabbit red blood cells were used in place of fresh sheep red blood cells. The antigen complex was diluted so as to give a 1.25% solution of red blood cells instead of the prescribed 2.5%. The sensitizing agent was insulin. The diluent was 1:100 normal rabbit serum in 0.85% saline. Serial dilutions of serum up to 1:5120 were used. All the tests were run at room temperature and the settling patterns read after 2 h. Control tanned formalinized, and plain formalinized rabbit red blood cells were tested against 1:100 normal rabbit serum (hemagglutination diluent) and with 1:10 mouse serum from each mouse used in the experiment.

The results of these tests showed no difference between KL tolerant and BUB sensitive mice. Controls with rabbit serum were negative and positive reactions observed with mouse sera were due to an agglutinin for rabbit red cells. Antibodies against insulin were therefore not detected in any mouse.

Blood sugar levels of BUB mice were determined after injecting plasma of KL mice which had received various types of treatment, as indicated in the Table. BUB mice were fasted for 3 h, after which a blood glucose determination was performed to obtain a normal blood glucose level. Following this, the animals were given food ad libitum, and test injections were given. For the tests of blood sugar, blood obtained from the orbital sinus was centrifuged at 2000 rpm for 10 min. The plasma was then used for determination of the glucose level by the Glucostat Enzyme Colorimeter method. When more than one animal was used for a test, the values for blood glucose levels were averaged.

As indicated in the Table, serum of KL mice injected into the test animals produced a slight elevation of blood glucose. Serum of KL mice injected 2 h previously with insulin produced a lowering of the blood sugar. Plasma incubated with insulin in vitro and then injected also caused a fall in blood sugar. Plasma of a KL animal administered to a BUB did not protect the animal from the hypoglycemic effect of insulin injected later.

These data indicate that the insulin tolerance of the KL mice cannot be explained as being due to antibodies to the insulin and that with the methods used, no indication was found of binding of the insulin in an inactive form[6].

*Zusammenfassung.* Die Serum-Eigenschaften eines gegen hohe Insulindosen resistenten Mäusestammes KL werden mit einem Stamm BUB verglichen, bei welchem bereits eine zehnfach kleinere Insulindosis Konvulsionen verursacht. Es handelt sich nicht um einen Serumfaktor des Stammes KL, der die Insulinresistenz bewirkt oder der den Stamm BUB schützt. Da keine Antikörper gefunden wurden, bleibt die Ursache der Resistenz unaufgeklärt.

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[5] A. B. STAVITSKY, J. Immun. *72*, 360 (1954).

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## The Effect of Phytohemagglutinin in vivo on the Mitotic Activity of the Bone Marrow Cells in Young Rats

DE VRIES and VAN WENT[1] used heterologous human O serum to stimulate mitoses in bone marrow cells for chromosome investigations, postulating that the action of the serum might be analogous to hetero-antibody reactions.

Phytohemagglutinin (an extract of the red kidney bean *Phaseolus vulgaris*) in its 'P' form is a protein which agglutinates red blood cells and has a mitogenic action on white blood cells. It is commonly used in cytogenetic studies to stimulate the transformation of white cells into blast-like forms which then divide mitotically. Recently, HUMBLE[2] claimed increased hemopoiesis after injecting it into aplastic anaemia patients, but FLEMING[3] and RETIEF et al.[4] were not able to confirm this.

The present investigation was designed to show the stimulating effect of phytohemagglutinin 'P' (Difco) on bone marrow cells of rats of different age groups.

*Material and methods.* 26 Wistar type rats in age groups of 3, 6, and 12 weeks, and with equal numbers of males and females in each group, were used experimentally. Comparable groups for number, age, and sex were used as controls. Each experimental animal received 0.5 ml reconstituted phytohemagglutinin 'P' (Difco) intraperitoneally, followed by 1 ml of 0.01% solution of 'Colcemid' (Ciba) 22 h later. The animals were killed after $5\frac{1}{2}$ h and a femur quickly removed. The upper and lower epiphyses were cut off and the marrow flushed out with a syringe and fine needle, using 0.8% aqueous sodium citrate. The volume was made up to 2 ml and the whole incubated for 20 min at 37°C. After centrifugation at 500 rpm the supernatant fluid was decanted and the pellet of cells dispersed by gentle shaking in the remaining fluid. Fixative, 2 ml of a 3:1 ethyl alcohol/acetic acid mixture, was added drop by drop down the side of the tube with gentle agitation between drops. The suspension was refrigerated for $\frac{1}{2}$ h at

[1] G. F. DE VRIES and J. J. VAN WENT, Stain Technol. *53*, 39 (1964).

[2] J. G. HUMBLE, Lancet *1964 i*, 1345.

[3] A. F. FLEMING, Lancet *1964 ii*, 647.

[4] F. P. RETIEF, H. P. WASSERMAN, and N. G. HOFMEYER, Lancet *1964 ii*, 1344.

4°C, recentrifuged, and the cells resuspended in fresh fixative before making slide preparations by the method of BISHUN et al.[5] for human cells.

*Results.* Mitotic indices were calculated for each culture as the percentage of cells with visible chromosomes per 500 cells counted. The indices for each age group were averaged and the averages for the experimental and control animals compared. The average mitotic index of the 3-week-old animals treated with phytohemagglutinin 'P' was 9.8 as compared with 2.1 in the controls; that of 6-week-old animals was 6.0 as compared with 1.7 in the controls; and that of 12-week-old animals 8.9 as compared with 6.3 in the controls (Table).

The average mitotic index in each of the two younger groups was considerably higher (about four times) than that of the untreated control animals of comparable ages, while that of the older animals was not appreciably different in the treated and untreated animals. The numbers are too few for statistical analysis. There is no clear sex difference apparent except perhaps in the older treated females, four of which showed markedly higher mitotic rates than did the males of their subgroup.

There does seem to be a definite increase in the mitotic rates for the younger animals. This might be due to a more susceptible marrow in these groups than in the older animals. If this is so it might be possible to increase bone marrow activity in young individuals by phytohemagglutinin injections while impossible in older individuals.

This study is being extended to cover a larger number and greater age range of rats, and also of other animals, to see if the action is similar in other species[6].

Mitotic indices of control (C.) and experimental (Exp.) rats at 3, 6, and 12 weeks of age

| Age | 3 weeks | | 6 weeks | | 12 weeks | |
|---|---|---|---|---|---|---|
| | C. | Exp. | C. | Exp. | C. | Exp. |
| Females | 3 | 24 | 2 | 13 | 3 | 15 |
| | 2 | 6 | 2 | 9 | 7 | 14 |
| | 2 | 10 | 3 | 7 | 8 | 10 |
| | – | – | 1 | 3 | 12 | 15 |
| | – | – | 0 | 5 | 5 | 6 |
| Males | 2 | 8 | 1 | 5 | 2 | 5 |
| | 2 | 1 | 2 | 3 | 15 | 7 |
| | 2 | 10 | 1 | 2 | 4 | 6 |
| | – | – | 3 | 5 | 3 | 7 |
| | – | – | 2 | 5 | 4 | 4 |
| Average mitotic indices | 2.1 | 9.8 | 1.7 | 6.0 | 6.3 | 8.9 |

*Résumé.* Chez des rats (mâles et femelles) auxquels on a administré de la phytohémagglutinine P (Difco) par voie intrapéritonienne, l'activité mitotique dans les cellules de la moelle des os a été notablement plus grande à l'âge de trois et six semaines qu'à l'âge de douze semaines (par comparaison avec les animaux de contrôle).

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*Department of Anatomy, Queen's University, Belfast (Northern Ireland, U.K.), May 6, 1965.*

[5] N. P. BISHUN, W. R. M. MORTON, and B. MCLAVERTY, Lancet *1964 ii*, 315.

[6] We would like to thank Dr. J. B. BRIDGES of this Department for supervising the experimental work.

## Neurosecretory Cells in *Artemia salina* L.

It is well known that physiologically active substances are produced by the neurosecretory cells located throughout the nervous system of crustaceans[1]. These neurosecretory cells are distributed as distinct groups at least in the eye stalk and the brain. Relatively little is known about the neurosecretory system of anostracans[2,3], a primitive group of crustaceans. By analogy with what obtains in many other crustaceans the neurosecretory organs of which have been recently investigated, control of many metabolic processes in the Anostraca may be expected to be under hormonal control originating in the neurosecretory cells.

With this end in view, we have studied *Artemia* collected from the Sambhar salt lake, Rajasthan. The neurosecretory groups of cells have been identified by using the Gömöri technique. Large neurosecretory cells are seen in clusters in the supraoesophageal ganglion and in the eye stalk. Based on the shape, presence or absence of vacuoles in the cytoplasm and on the nature of secretion, the neurosecretory cells may be classified into three groups. One type of cell is large (15–20 $\mu$), oval in shape with vacuolated cytoplasm and large nuclei (Figure 1). The cytoplasm of these cells is basophilic with the Nissl substance zonated. The nuclear membrane also stains basophilic, but the nucleoplasm is acidophilic. Some of these cells show large axons. The secretory granules occur in aggregates in the nucleus and also outside the Nissl zone. The second type of cell (Figure 1) is relatively small (8–12 $\mu$) with little cytoplasm and large spherical nuclei. These are without axons. While simulating the large cells in the secretory granules, these cells also show perinuclear concentration of neurosecretory granules. No tract from these is traceable into the eye stalk. Both these types of cells are seen in the supraoesophageal ganglion. A third type is present in the X-organ of the eye stalk (Figure 2). These cells are small (3–5 $\mu$) and form grape-like clusters with practically no cytoplasm.

[1] D. B. CARLISLE and F. G. W. KNOWLES, *Endocrine Control in Crustaceans* (Univ. Press, Cambridge 1959).

[2] J. H. LOCKHEAD and R. RESNER, Proc. 15th Int. Cong. Zool. *4*, 397 (1958).

[3] M. MENON, *Neurosecretory System of Streptocephalus sp. (Anostraca: Branchiopoda). Neurosecretion*, Mem. Soc. Endocrin. (Eds. H. HELLER and R. B. CLARK; Acad. Press, London 1962), p. 411.