since it occurs before the onset of the dorsal root potential that can be evoked from the red nucleus.

Recording of the extracellular field potential that is evoked in the spinal cord from the red nucleus shows a monosynaptic focal potential in Rexed's layer VI and VII, which indicates that the rubrospinal tract has monosynaptic connections with neurones in this region. This is illustrated in Figure 2 by the left records showing a maximal monosynaptic focal potential at a depth of 2.5–2.6 mm. For comparison it is shown in the corresponding right records that the focal potential evoked monosynaptically from group I muscle afferents is maximal at a depth of 2.2 mm, which is in the intermediate nucleus in layer VI[6]. Extra- and intracellular recording from interneurones in the dorsal horn and intermediate region has revealed excitatory action from the red nucleus on interneurones monosynaptically activated by Ia or Ib afferents. Among the interneurones activated from the FRA, two types are found: some interneurones are facilitated, others are inhibited from the rubrospinal tract. In layer VII, ventral to the intermediate nucleus, interneurones are found that can be excited from the red nucleus but not from primary afferents. It does not seem likely that the strong excitatory action that is evoked from the rubrospinal tract in flexor motoneurones can be explained by the excitation of interneurones in reflex pathways. It is more likely that flexor excitation is mediated via those interneurones in layer VII that are activated from the rubrospinal tract but not from primary afferents.

The importance of the supraspinal control of transmission in reflex pathways for motor regulation is emphasized by the finding that facilitation through excitatory action on interneurones of reflex paths can be evoked both from the corticospinal and rubrospinal tracts. It is postulated that the rubrospinal action is governed from cerebellum.

Résumé. La stimulation du Noyau Rouge a pour effet une facilitation des interneurons intercalés entre des afférences primaires et les motoneurons et provoque dans les motoneurons des potentiels postsynaptiques bisynaptiques.

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[6] J. C. ECCLES, P. FATT, S. LANDGREN, and G. J. WINSBURY, J. Physiol. *125*, 590 (1954).

Tests for an Anti-Insulin Factor in the Plasma of Insulin-Tolerant Mice

Mice of the selected inbred KLC57 Black strain can tolerate from 200 to 500 units of insulin without convulsing, while mice of the BUB strain convulse with much smaller amounts, usually 20 units or less[1–3]. In a preliminary study, SNEDECOR[4] found that serum from mice of the tolerant strain mixed with insulin in vitro produced a lowering of the blood sugar when injected into a non-tolerant mouse. Further study of the tolerant and non-tolerant strains was undertaken to see if differences between the strains could be detected in hemagglutination reactions as an indication of possible antibodies to insulin, and to determine, by means of bioassay, insulin activity after contact of the insulin with serum of tolerant animals in vivo and in vitro.

KL and BUB strains of mice were derived from insulin tolerant and insulin sensitive strains at Brown University and have been maintained as inbred strains at the University of Rhode Island since 1960. The mice were maintained in special animal quarters at 77°F, 50% humidity, and 12 h of light per day. Purina Laboratory Chow and water were available ad libitum. Each KL animal was tested at 40 days of age with 200 units of insulin (except in Groups B and C, Table); surviving animals were used for matings and experiments. All insulin used was Iletin (Lilly) purchased as U 40 or U 500 preparations. All injections were intraperitoneal. All tests were run between 9 p.m. and 3 a.m. A total of 28 KL and 38 BUB mice was used.

For hemagglutination tests, blood obtained from the sub-orbital sinus by means of a capillary pipette was allowed to remain at room temperature until clotting began and was then refrigerated for 18–20 h. The blood clot was removed and the plasma remaining was centrifuged at

In vivo assay

Test	Description of injections	Number of BUB animals	Time after injection, min	Blood sugar (average) mg%
Insulin control	10 U insulin	2	0	125
			20	31
			40	20
			70	15
			110	moribund
Plasma control	0.5 ml KL plasma	2	0	100
			20	125
			40	125
			70	125
			110	125
Test A	0.5 ml plasma of KL mice injected with 40 U insulin 2 h previously	4	0	120
			25	40
			50	dead
Test B	0.5 ml KL plasma; challenge with 10 U insulin 5 min later	3	0	120
			35	40
			60	20
			90	dead
Test C	0.5 ml KL plasma treated with 10 U insulin in vitro for 10 min	3	0	100
			20	40
			45	40
			75	20
			115	dead

[1] H. B. CHASE, M. S. GUNTHER, J. MILLER, and D. WOLFFSON, Anat. Rec. *99*, 678 (1947).
[2] B. F. ARGYRIS, Endocrinology *64*, 400 (1959).
[3] E. B. CHASE and D. K. CARRIER, Am. Zool. *3*, 539 (1963).
[4] J. G. SNEDECOR, Anat. Rec. *113*, 589 (1952).

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.