In order to determine the specificity of these calcium effects, salts of various cations were tested. In all experiments, 0·03 ml. of 0·1 M salt solutions and 0·1 ml. of substrate, plasma or 0·3 per cent of bovine fibrinogen, and of clotting factor were used. Chlorides of sodium, potassium, magnesium, calcium, strontium, and barium were added to oxalated plasma and clotting factor. Strontium and barium chloride shortened the control clotting time of 25 sec by 7 sec, calcium chloride by 11 sec; the other salts left it unchanged. With fibrinogen as substrate none of the salts had an accelerating effect; however, calcium, strontium, and barium chloride eliminated the delay in clot formation brought about by sodium oxalate[3] and its nearly complete inhibition by sodium citrate.

In other tests the three salts exhibited different modes of action. Salts of strontium, like calcium salts, produced an immediate turbidity when added to oxalated plasma and clotted it directly, while with barium salts there was no visible change. The chloride, bromide, nitrate, and acetate salts of strontium, respectively, were used. The clotting time varied from 10 to 20 min for plasma which would coagulate in 3–4 min with calcium chloride. The resulting clots were insoluble in urea.

In a reaction mixture of precipitated barium oxalate, fibrinogen, and clotting factor clearing occurred, while with calcium or strontium oxalate there was no change. This could be due to the formation of a complex compound, which would also explain the failure of barium chloride to react visibly with oxalated plasma.

The stability of clotting factor-fibrinogen clots was unaffected by the addition of barium chloride. They dissolved in 30 per cent urea in about 1 h; clots with strontium chloride required 3–4 h and clots with calcium chloride 8–10 h for dissolution.

Potent preparations of clotting factor[3] contained 17 μg of calcium per ml., as determined by flame photometer measurements. Oxalate and citrate most likely enter into combination with the factor through calcium and are, in turn, readily removed by precipitating cations. Under physiological conditions of blood clotting, the amount of clotting factor produced in a certain volume of plasma would seem to be just sufficient to coagulate it. The anticoagulant effect of oxalate or citrate added in the prescribed quantities would then be due to depression of clotting factor activity, not to interference with clotting enzyme formation. Neither oxalate nor citrate destroys clotting factor. There was no change in factor activity even after 48 h incubation at 37° C with the anions.

In the stabilization of fibrin clots, strontium apparently can take the place of calcium, though it may not fit as well into the structure of the fibrin molecule. It is possible that this is the mechanism by which strontium is deposited in tissues in the calcification process.

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Effects of Phytohæmagglutinin *in vivo* in Rats

Several reports[1–4] during the past three years have shown that phytohæmagglutinin (PHA) (a muco-protein derived from the red kidney bean, *Phaseolus vulgaris*) possesses mitosis-stimulating properties on lymphocytes in short-term culture. Despite the considerable interest existing concerning the nature of this drug, there is no information available on its effect *in vivo* apart from a report by Goddard and Mandel thirty-four years ago[5]. For this reason the following investigation was undertaken.

Young female Wistaria rats of approximately the same age (8 weeks) and weight (50 g) were grouped as follows: I (4 rats), saline treated controls; II (4 rats), PHA 0·2 ml. × 4 at weekly intervals subcutaneously; III (4 rats), PHA 0·2 ml. × 4 at weekly intervals intraperitoneally; IV (4 rats), PHA 0·2 ml. intraperitoneally followed by 3 weekly doses of 0·1 ml. subcutaneously; V (2 rats), PHA 0·5 ml. intraperitoneally followed by 3 weekly doses of 0·1 ml. subcutaneously respectively.

The PHA used throughout was the Difco *M* form. During the four weeks of observation the rats were weighed at weekly intervals, and packed cell volumes, white cell counts and differentials carried out on tail-vein blood at the same time. At the end of this period, two rats in each group were killed and post-mortem examinations performed.

All rats in each group gained from 5 to 10 g in weight during the experiment. Their packed cell volumes remained within the range 38–48 per cent for all animals with no difference between the control and any treated group. The white blood cell counts were also probably within the rather wide range of normal values, with differential counts of 68–90 per cent lymphocytes, without increase in the treated groups.

Normal values for the rat are given as: packed cell volume (adult) 50 per cent; total white blood count 6–18,000/mm³; differential count (lymphocytes) 55–96 per cent[6].

Serum globulin determinations at the time of death were as follows: I, 0·81 and 0·95 g per cent; II, 1·16 and 0·97 g per cent; III, 0·62 and 0·90 g per cent; IV, 0·62 and 0·84 g per cent; V, 0·85 and 0·53 g per cent.

The rat spleens and livers were weighed with doubtful differences between control and treated groups as shown in Table 1.

Table 1.

Group	Spleen weight (g)	Liver weight (g)
I	0·75	7·8
	0·80	7·35
II	0·70	8·75
	0·75	7·95
III	1·05	8·75
	0·95	9·45
IV	0·98	10·20
	1·05	9·30
V	0·82	9·35
	0·96	9·95

Histological examination of the spleens showed a possible 'cuffing' of pale, large lymphocytes around the follicles in some of the treated rats (groups II, III and IV), but it was impossible to establish a consistently abnormal pattern.

No local lesions or tumours at the sites of any injections were noted and no enlargement of lymph glands or other signs of lymphatic stimulation seen.

From these results it may therefore be concluded that, in the doses given, PHA is not toxic to rats, does not produce active hæmolysis or observable agglutination of the red cells, and its ability to produce lymphoproliferation such as is seen *in vitro* in lymphocyte culture could not be unequivocally confirmed.

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