to enhance insulin release; mean secretion was 35 ± 9 μU/ml. However, glucagon promoted a significant increase in insulin secretion (70 ± 12 μU/ml), even when we allowed for maximum possible contamination of the glucagon with insulin. The results of analysis of variance and Duncan's new multiple range test indicated that insulin output from the group with glucagon was significantly greater than that of the other groups ($P < .01$).

The supply of viable fetal pancreatic tissue is extremely limited, and thus the number of experiments performed is by necessity small. Our results, although limited in number, indicate that isolated pancreatic islets, obtained from human fetuses of an early gestational age, do not respond to either glucose or tolbutamide. This observation could lead one to doubt the viability of the preparations, were it not for the significant insulin release promoted by glucagon.

Milner and Wright (*7*) were among the first to report that in human newborns intravenous administration of glucagon directly stimulated insulin release. Similar observations that glucagon in the absence of glucose can stimulate insulin secretion had been made previously with rat pancreatic slices (*8*) and with isolated perfused rat pancreas (*9*), as recently reviewed by Mayhew *et al.* (*10*). Therefore, our observation that glucagon directly stimulates pancreatic insulin release is not an isolated finding. It is interesting, however, that glucagon did promote insulin secretion when islets obtained from the same pancreas failed to respond to either glucose or tolbutamide. Failure to observe glucose-induced insulin release in vivo has been reported for the fetus of the sheep (*11*) and the subhuman primate (*12*), and for pancreatic slices from the fetal rat (*13*) and 24-day-old rabbit fetus (*14*). On the other hand, Correa *et al.* reported that pancreatic slices taken from fetal calves less than 5 months old responded to glucose (*15*).

The lack of response to glucose by the fetal pancreas apparently disappears during or shortly after delivery (*16*), although it may persist in the premature infant (*17*). In the newborn a definite, although occasional, sluggish insulin response to glucose has been well documented (*16–18*). The age spectrum of pancreatic response to glucagon contrasts interestingly with that to glucose and tolbutamide; and, as shown here in human fetal islets preparations, glucagon alone possesses the capacity to promote insulin release during critical stages of fetal development.

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Inhibition of Cytotoxicity of Lymphocytes by Concanavalin A in vitro

Abstract. *Human lymphocytes treated with the plant protein concanavalin A are stimulated to transform into blasts, without developing cytotoxicity for chicken erythrocytes. Prior treatment of lymphocytes with concanavalin A potentiated phytohemagglutinin-induced blast transformation and DNA synthesis but completely inhibited phytohemagglutinin-induced cytotoxicity. Inhibiton was not due to suppression of the mixed lymphocyte–erythrocyte aggregation normally caused by phytohemagglutinin. Inhibition of cytotoxicity was reversible when concanavalin A was removed from the lymphocytes by treatment with methyl-α-D-mannopyranoside after 1 hour but not after 20 hours. The results indicate that blast transformation and cytotoxicity are separate expressions of lymphocyte stimulation.*

Lymphocytes of human or animal origin are cytotoxic to tissue culture cells or chicken red blood cells (that is, target cells) after stimulation with phytohemagglutinin, antigen, allogenic lymphocytes, or certain antibodies to target cells. The reactions are related to the immunospecific cytotoxicity produced by lymphocytes from donors sensitized to target cell antigens and are believed to constitute models in vitro for cell-mediated tissue damage in vivo (*1*). When cytotoxicity is induced by stimulation of lymphocytes, the strength of the cytotoxic reaction is correlated to the incidence of blast transformation induced by the stimulating agent (*2*). However, cytotoxicity is assumed to be an expression of an energy requiring activation of the lymphocytes rather than of blast transformation per se (*2*, *3*). We report here that treatment of lymphocytes with the plant protein concanavalin A (conA) (*4*) suppresses cytotoxicity but not transformation, providing new evidence for the independence of these phenomena.

Concanavalin A was extracted from commercial jack bean meal (*Canavalia ensiformis*) and purified as described (*5*, *6*). It was dissolved in 0.1*M* phosphate buffer, *p*H 7.9, to give a stock solution of 800 μg/ml. Lymphocytes

were isolated from defibrinated human blood by gelatin flotation (*7*). They were further purified by incubation at 37°C for 30 minutes on a column packed with nylon wool (*8*) and subsequent elution with four volumes of tris buffered Hanks balanced salt solution, *p*H 7.4 (TH), containing 5 percent fetal calf serum (*8*). In the experiments to be described, 98 to 99.5 percent of the leukocytes were lymphocytes. Chicken erythrocytes were isolated from heparinized blood and were labeled with sodium [^{51}Cr]chromate (*8*, *9*).

For the cytotoxicity experiments, 2.5 $\times$ 10^6 appropriately treated and viable lymphocytes were suspended in 0.5 ml of Parker's medium 199 supplemented with 5 percent heat-inactivated (60 minutes, 56°C) fetal calf serum, glutamine, penicillin, and streptomycin (*8*). They were then mixed with 0.5 ml of medium containing 10^5 labeled chicken erythrocytes. Where indicated, phytohemagglutinin (*8*) was immediately added in 0.5 ml of the same medium. In lymphocyte-free controls, 20 $\times$ 10^6 unlabeled chicken erythrocytes were added in order to keep the cell concentration sufficiently high. The cell mixtures were incubated at 37°C (in duplicate) under tissue culture conditions for various intervals. At the end of the experiments, cells and supernatant were separated by centrifugation at 1500 rev/min for 10 minutes, and radioactivity was determined in a well-type scintillation counter. Cytotoxicity was measured by radioactivity in the supernatant and is given as the percentage of total radioactivity in the incubation mixture. Independent duplicates almost never varied by more than 2 percent in regard to isotope release (*3*, *9*). A release of 85 to 90 percent of the isotope from cells to supernatant is equivalent to 100 percent lysis (*10*).

For determination of DNA synthesis in stimulated lymphocytes, 1.25 $\times$ 10^6 cells were incubated for 48 hours in a total volume of 1.5 ml of Parker's medium containing 15 percent heat-inactivated human serum. [^{14}C]Thymidine (0.2 μc) (*8*) in 0.1 ml of TH were then added to each tube. After the mixture was incubated for 16 hours more, DNA was processed on filter paper disks (*11*), and its radioactivity was determined by liquid scintillation counting. For microscopic observations, mixtures of 2.5 $\times$ 10^6 lymphocytes and 5 $\times$ 10^5 chicken erythrocytes were incubated for various intervals in 3 ml of medium. Portions (0.15 ml) were sedimented on microscopic slides by centrifugation for 15 minutes in a cytocentrifuge. The slides were stained with May-Grünwald-Giemsa stain.

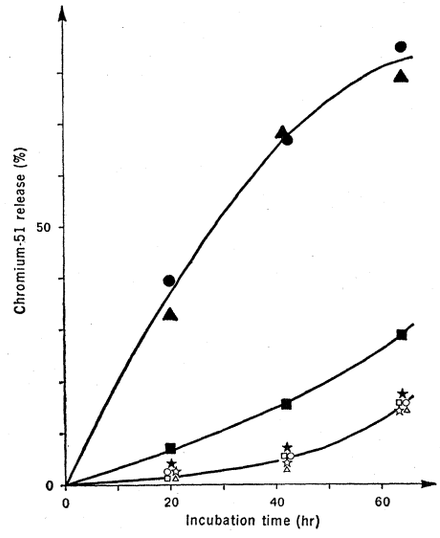

Fig. 1. Percentage of ^{51}Cr release after different periods of incubation. Filled symbols, incubation mixtures containing phytohemagglutinin. Empty symbols, no phytohemagglutinin. Circles, lymphocytes without conA. Squares, lymphocytes treated with conA. Triangles, lymphocytes first treated with conA and subsequently with methyl-α-D-mannopyranoside. Asterisks, lymphocyte-free controls. All points are the means of duplicate incubations.

We first tried to find out whether or not conA, known to be a potent lymphocyte stimulating agent (*12*, *13*), also would induce the lymphocytes to become cytotoxic to chicken erythrocytes. Lymphocytes (2.5 $\times$ 10^6 per tube) were suspended in 0.5 ml of Parker's medium without serum in order to prevent neutralization of conA by serum glycoproteins (*6*, *13*, *14*). Dilutions (0.5 ml) of conA in TH were added to the tubes which were then incubated for 30 minutes at 37°C in an atmosphere of 95 percent air and 5 percent CO_2. Control lymphocytes were treated similarly but without conA. The conA concentrations tested ranged from 12.5 to 200 μg per tube. The cells were washed once with 1 ml of TH containing 5 percent fetal calf serum and were then mixed with chicken erythrocytes as described. After 24 hours of incubation, no cytotoxic effect was observed. Isotope release from the red cells was 4 to 6 percent in all tubes including the controls. The viability of the lymphocytes was unimpaired, as assessed by trypan blue exclusion. At the concentrations used, conA stimulated blast transformation and enhanced DNA synthesis.

The results suggested either that lymphocytes stimulated by conA represent a population of cells distinct from the cytotoxic cells produced by phytohemagglutinin or other stimulants or that conA inhibits the cytotoxicity of stimulated lymphocytes in a more general fashion. To decide between these alternatives, we mixed conA-treated lymphocytes with chicken erythrocytes in the presence of phytohemagglutinin. At a dose range of 12.5 to 200 μg per tube, conA strongly inhibited phytohemagglutinin-induced cytotoxicity (ten experiments).

Table 1 shows the typical results of one of two independent experiments in which cytotoxicity and stimulation of DNA synthesis were studied in parallel with a constant dose of conA and phytohemagglutinin (50 and 20 μg per 2.5 $\times$ 10^6 lymphocytes, respectively). While the cytotoxicity induced by 20 μg of phytohemagglutinin was completely suppressed, DNA synthesis was enhanced. Optimum doses of phytohemagglutinin have been shown to be 20 to 50 μg (*2*, *9*) for expression of cytotoxicity. Inhibition of cytotoxicity by conA was not overcome by increasing these doses of phytohemagglutinin. Similar results were obtained when lymphocytes were first treated with phytohemagglutinin for 30 minutes before addition of conA. Thus, conA inhibits phytohemagglutinin-induced lymphocyte cytotoxicity without interfering with blast transformation or DNA synthesis. The results are the opposite of those obtained with antimetabolites which inhibited protein or nucleic acid synthesis of phytohemagglutinin-stimulated lymphocytes without affecting cytotoxicity (*3*, *15*).

Antilymphocyte serum which also stimulates lymphocytes suppresses phytohemagglutinin-induced cytotoxicity (*16*). Both light microscopy and electron microscopy have yielded results suggesting that antilymphocyte serum inhibits phytohemagglutinin-induced cytotoxicity, at least in part, by suppressing the mixed aggregation normally seen between lymphocytes and target cells (*17*). The mode of conA inhibition seems to be of a different nature. Incubation of conA-treated lymphocytes with phytohemagglutinin and chicken erythrocytes leads to a mixed aggrega-

Table 1. The effects of conA on cytotoxicity and DNA synthesis of lymphocytes. Cytotoxicity was measured after 20 hours of incubation. All numbers in the table are the means of duplicate incubations. DNA synthesis is expressed as count/min per tube. PHA, phytohemagglutinin.

Incubation mixtures	^{51}Cr-release (%)	DNA synthesis (count/min)
Untreated lymphocytes	1.5	192
Untreated lymphocytes, PHA	39.6	31,587
ConA-treated lymphocytes	1.7	17,861
ConA-treated lymphocytes, PHA	6.2	84,097
No lymphocytes	2.0	
No lymphocytes, PHA	3.0	

tion as pronounced as that obtained with phytohemagglutinin alone. However, whereas the chicken erythrocytes in these aggregates are heavily damaged in the experiments with phytohemagglutinin alone, they appear undamaged and present in large numbers even after 3 days of incubation in the experiments with conA-treated lymphocytes. The results suggest that no microscopically visible change of the aggregation pattern is responsible for conA-induced inhibition of cytotoxicity. This does not rule out the possibility that conA exerts its inhibitory action primarily by affecting surface-bound activities of the lymphocytes.

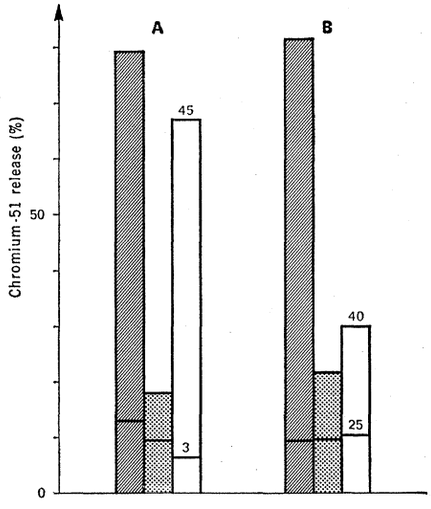

Fig. 2. Percentage of ^{51}Cr release after exposure of erythrocytes to lymphocytes and phytohemagglutinin. The horizontal lines near the bottom show ^{51}Cr release in the phytohemagglutinin-free controls. The ^{51}Cr release in lymphocyte-free controls was ≤ 6 percent in all samples. The hatched columns show phytohemagglutinin-induced ^{51}Cr release obtained with untreated lymphocytes; dotted columns, with conA-treated lymphocytes; empty columns, with lymphocytes from which conA was removed by methyl-α-D-mannopyranoside 1 hour (A) or 20 hours (B) after addition of conA, respectively. The numbers in the diagram give the percentage of transformed lymphocytes seen in cytocentrifuge preparations of samples free of target cells and incubated as indicated for the cytotoxicity tubes.

Concanavalin A combines with certain carbohydrate residues on proteins or cell surfaces and simple carbohydrates can be used to reverse these interactions (*4*, *13*, *18*). Methyl-α-D-mannopyranoside is one of the most potent inhibitors of the interaction of conA with polysaccharides (*19*). Figure 1 shows an experiment in which lymphocytes were allowed to interact for 30 minutes with 50 μg conA per 2.5×10^6 cells as described. After centrifugation, some of the samples were extracted twice for 10 minutes at 37°C, with two 1-ml portions of 0.1*M* methyl-α-mannopyranoside in TH, or with TH only. A cytotoxicity experiment was then performed in the usual manner (50 μg of phytohemagglutinin per tube). During the 64 hours of incubation, control lymphocytes plus phytohemagglutinin led to complete lysis of the red cells. Treatment with conA inhibited phytohemagglutinin-induced cytotoxicity, corrected by subtracting ^{51}Cr release in the controls (*20*), by more than 80 percent throughout the entire course of the experiment. Removal of conA by the methyl-α-D-mannopyranoside after 30 minutes completely abolished this inhibition. Four independent experiments of this type gave similar results.

It could be assumed that conA exerts its inhibitory effect by blocking lymphocyte effector sites necessary for the cytotoxic reaction. However, the situation may be more complicated. In the experiment shown in Fig. 2A, the lymphocytes were first incubated for 1 hour in serum-free medium with or without 50 μg of conA. Part of the lymphocytes were then extracted with methyl-α-D-mannopyranoside. Chicken erythrocytes and 50 μg of phytohemagglutinin were added after washing, and cytotoxicity was assayed after 44 hours of incubation. In Fig. 2B, portions of the same conA-treated or -untreated lymphocytes were incubated for an additional 19 hours in 2.5 ml of medium (containing 15 percent of normal human serum) before the methyl-α-D-mannopyranoside was added. Erythrocytes and phytohemagglutinin were incubated for 44 hours as in the experiment of Fig. 2A. Removal of conA by methyl-α-D-mannopyranoside after 1 hour of incubation reversed inhibition of phytohemagglutinin-induced cytotoxicity by 90 percent. In contrast, inhibition was more or less irreversible (that is, 70 percent inhibition) when the sugar was added after 20 hours of conA treatment. Cells extracted with the sugar after having been in contact with conA for 20 hours, and then incubated with chicken erythrocytes in the absence of phytohemagglutinin, were not cytotoxic in spite of a high incidence (25 percent) of blast transformation. Removal of conA after 1 hour, in the absence of phytohemagglutinin, reduced blast transformation to 3 percent (*13*).

Several explanations may account for these findings. It cannot yet be excluded that prolonged treatment with conA selectively inactivates or kills a fraction of the cell population which may be essential for executing the lytic reaction but which does not undergo blast transformation. Alternatively, conA may cause some functional changes of activated and transforming effector cells which thereby lose their ability to develop cytotoxicity.

The inhibition of lymphocyte cytotoxicity by conA is a general phenomenon. Thus, conA inhibits the cytotoxic effects of lymphocytes from normal humans against antibody-coated target cells of different kinds, or those of lymphocytes from immunized animals against antigenic target cells (*21*). It has also been reported that conA has immunosuppressive effects in vivo on the rejection of skin allografts in mice (*22*) and blocks expression of delayed hypersensitivity to tuberculin in guinea pigs (*23*). However, conA stimulates normal guinea pig lymphocytes to produce a nondialyzable factor producing skin lesions resembling those of delayed hypersensitivity (*24*). It remains to be seen whether immunosuppression by conA in vivo and suppression of cytotoxicity in vitro are related phenomena.

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Chromosomal Drift, a New Phenomenon in Plants

Abstract. *A seasonal shift in chromosome number of reproductive cells of* Claytonia virginica, *which coincided with near-drought conditions, resulted in a chromosome complement new to the population. The number reappeared 2 years later, the minimum time required for plants of that generation to produce flowers. Moreover, the chromosome number of root cells commonly differed from aerial organs of the same plant.*

Diversity of chromosome number is widespread within certain plants. Its frequency is becoming more apparent, especially among annuals and herbaceous perennials, as more than one individual from each of several populations is studied. One example of such diversity is found in spring beauty *Claytonia virginica* L. in which 50 known cytotypes for the species range in diploid chromosome number from 12 to about 191 (*1*).

Although infraspecific aneuploidy and polyploidy are well recognized, no long-range study has included the relation of the effects of time on chromosomal variability within a single population. A micropopulation of *C. virginica* from the vicinity of Carthage, Panola County, Texas (*2*), was studied during four annual flowering seasons over a span of 8 years. The locality is about 20 m^2 with a gradual rise of 2 m near the middle. On this knoll, plants flower first at the top in late February and gradually flower down the slopes until the population completes flowering by the end of March or early in April. The population consists of perhaps 150 plants with about one-half in flower at any one time. From an underground stem (corm), one to several floral shoots emerge over a period of roughly 3 weeks depending on age and the environment. Plants grow largely during warm periods from autumn through winter and die back to the corm shortly after flowering. Results are based on samples averaging one-third of plants in flower at the time of sampling. Cells examined were those in meiosis in anthers unless noted otherwise.

In 1961, 1968, and 1969 chromosome numbers of these plants were similar (Table 1). In 1961 the study extended beyond the knoll population to include six other populations in the immediate area.

Yet in 1967 the chromosome number of these plants was different. In over two-thirds of them $2n$ was less than 28 and in 42 percent of these $2n$ was 24. I call this shift in chromosome number chromosomal drift (Fig. 1).

There is no ready explanation for this temporary change in 1967, but coincidentally one climatic factor varied during that season only. For the critical 6-month growing period roughly from November through April, rainfall was only 39.25 cm in the Carthage area, whereas, for the other 3 years, it varied from 76.25 to 97 cm which was about normal for a 20-year period. Is a precipitation of less than one-half the expected amount during a time of maximum growth and development responsible in some way for this marked chromosomal loss? Inasmuch as 42 per-

Table 1. Chromosome numbers of *Claytonia virginica* from the knoll population, vicinity of Carthage, Panola County, Texas.

Year	Plants (No.)	Chromosome number (as $2n$)																Chromosomes (av. No.)
		24	25	26	27	28	29	30	31	32	33	34	35	36	38	39	>39	
1961	15		1	1		1	4	3	2	2	1							29.6
1961*	61		2	1		21	18	5	5	3	3			2		1		29.6
1967	19	8	1	4		3	2			1								26.1
1967†	22					19	2	1										28.2
1968	23					6	7	7	2	1								29.4
1968†	7					6	1											28.1
1969‡	94	5			2	23	16	11	12	7	4	5	2	2	1	1	3§	30.6

* Plus data from six micropopulations in the vicinity of the knoll. † Mitosis from roots after transplanting to greenhouse. ‡ Based on three samples taken at 2-week intervals. § $2n = 41, 52, 58$.