jacks, solder was inserted, and the jacks were countersunk after the solder hardened. Now the blocks could be set onto the banana plugs on the panel and would close certain parts of the circuits involved.

Earlier machines by Stanhope (*3, 1*), Jevons (*3, 4*), Venn (*9*), Marquand (*6*), Pastore (*7*), and Macaulay (*5*) generally indicated the conclusions derivable from given premises. None could identify the fallacy in deductive thinking. However, in an article written in 1935 on the mechanical nature of problem solving, Hull (*2*) mentioned that he once constructed a "simple mechanism of sliding disk-segments of sheet-metal which will solve automatically, i.e., exhibit the conclusions logically flowing from all of the known syllogisms and which will automatically detect all of the formal fallacies . . ." but that ". . . a description has not yet been published."

The logic machine described by the present writer is an electrical device which identifies the formal fallacy (or fallacies) in an invalid syllogism of the categorical, hypothetical, or disjunctive types, or in conversion and obversion. It has been used successfully in logic classes and evokes sharp student interest. As with all such machines, its obvious limitation is that the "argument" must first be put into logical form by a human being.

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The Application of Soybean Inhibitor in Tissue Cultivation

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It is well known that certain types of tissue cells liquefy clots of homologous plasma, thus making their permanent cultivation impossible. Carrel and Ebeling (*1*) attempted unsuccessfully to prevent such liquefaction by adding traces of unsaturated fatty acids, egg yolk and other substances to the medium. The present author (*2*) cultivated permanent strains of the Rous sarcoma cells *in vitro* for many years by putting a small piece of boiled muscle tissue beside the tumor tissue. The dead muscle tissue served as a kind of solid substrate which was invaded by the sarcoma cells and thus it made possible the transfer of the cells from medium to medium. This method of course did not allow quantitative estimations of the rate of growth characteristic for the tumor cells. Later we found that sarcoma cells grow readily on certain heterologous media without liquefaction of the medium, probably because the chicken cells are unable

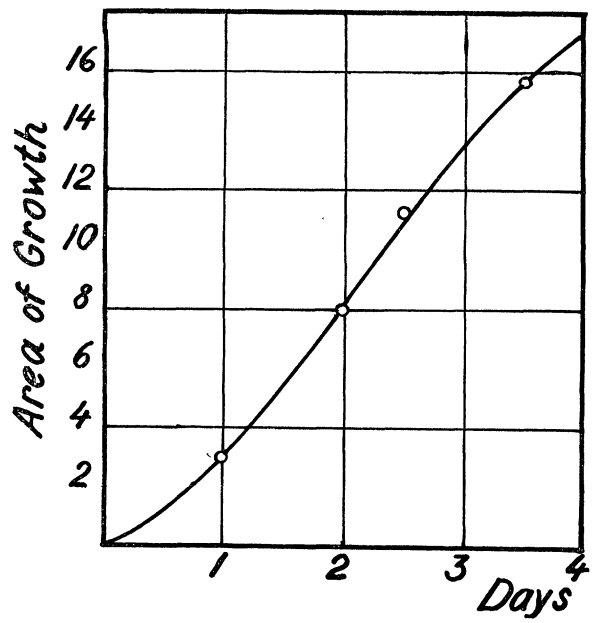

Fig. 1.

to activate the proteolytic enzymes in the heterologous plasma (*3*).

In this paper a method will be described for the cultivation of Rous sarcoma cells on clots of homologous plasma in which it was found that liquefaction could be prevented by an addition of the soybean inhibitor first isolated by Kunitz (*4*). In this manner it was possible to measure the rate of growth of the Rous chicken tumor cells in homologous plasma medium. The growth-retarding effect of soybean inhibitor on the growth of myoblasts will likewise be described and discussed.

The plasma medium in Carrel flasks was made up as follows: *Solid phase*—0.5 ml chick plasma, 1.0 ml Tyrode's solution, and 0.2 ml of a 0.35% solution of trypsin inhibitor; this was coagulated by adding 1 drop of embryo juice. *Fluid phase*—0.4 ml mixture of equal volumes of chick serum and Tyrode's solution, and 0.2 ml embryo extract.

Liquefaction of the medium was found to be completely prevented. Fig. 1 demonstrates the regular growth of the Rous sarcoma cells when cultivated in the presence of the inhibitor. The growth rate of the cells under the conditions of this experiment could now be accurately measured and was found to be relatively low. It therefore seems that the soybean inhibitor slows down the growth rate of the tumor cells.

Further it was found that the inhibitor prolongs the coagulation time of the plasma used. In those of our

experiments in which the inhibitor was added to the blood plasma and not to be supernatant fluid of the flask cultures the coagulation of the plasma was found to be considerably delayed or completely prevented, even when large amounts of embryo juice was added. These facts suggest that the inhibitor acts on the system involved in the coagulation of the blood plasma as well as on the proteolytic enzymes of the surface of the cells, the desmo-enzymes.

Recently Overman and Wright (*5*) have observed that the trypsin inhibitor decreases the thromboplastic activity of the blood. The study of the effect of the soybean inhibitor on the growth of the cells must therefore be limited to such concentrations of inhibitor as will still allow coagulation of the plasma medium to take place. When the inhibitor, instead of being added to the plasma directly, is introduced into the supernatant fluid of the culture medium, the growth-inhibiting effect is relatively less pronounced.

The figures below illustrate the relative growth inhibition of fibroblasts as a function of increasing amounts of a rather dilute solution of the inhibitor which has been *introduced into the plasma medium* before clotting:

0.17% inhibitor solution	$Q = \frac{\text{Control}}{\text{experiment}}$[1]
0.1 ml	1.01
0.2 "	1.43
0.3 "	2.24
0.35% inhibitor 0.2 ml	2.22

Thus when more than 0.3 ml of a 0.17% solution of the inhibitor is added to the plasma clot, no further suppression of the growth takes place. This seems to indicate that the proteolytic enzymes are completely inhibited by that concentration.

The following results show the effect of a 0.7% inhibitor *added to the fluid phase of the culture medium*:

0.7% inhibitor solution	$Q = \frac{\text{Control}}{\text{experiment}}$
0.25 ml	1.44
0.30 "	1.20
0.35 "	1.18

To establish a certain degree of growth inhibition of tissue cells in a Carrel flask the concentration of inhibitor has to be four times that necessary to obtain the same degree of inhibition when the inhibitor is introduced in the solid phase of the plasma clot.

Two main facts have been observed in this investigation. The soybean inhibitor has been shown to be an important tool in the tissue cultivation technique. An addition of small amounts of inhibitor to the plasma medium stabilizes the clot and makes it possible to cultivate on homologous media tissues with strong inherent proteolytic activity. Thus it is now possible to cultivate malignant cells indefinitely and to make quantitative estimations of the rate of their growth. It will also be possible now, we believe, to study differentiation phenomena *in vitro* by means of suppressing the growth of the cells and slowing down their migration rate.

[1] The tissue area increase is measured by means of a planimeter and expressed in square centimeters, the area being projected with the aid of an Edinger projector enlarging 20 times. Q is a measure for the inhibiting effect.

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Occurrence of Vitamin B_c Conjugase in Human Plasma

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The occurrence in yeast extract of an antianemic principle, different from pteroylglutamic acid, but releasing that substance after acid or enzymatic hydrolysis, was first demonstrated by Binkley, *et al.* (*1*). Pfiffner (*2*) isolated this compound and named it temporarily vitamin B_c conjugate.

In 1947 Schweigert and Pearson (*3*) studied the folic acid content of blood plasma and corpuscles in Mammalia and fowl, before and after treatment with an enzyme, takadiastase, releasing folic acid from its microbiologically inactive forms. They could find only minute amounts of free folic acid, but it seemed from their experiments that vitamin B_c conjugate was present in significant amounts.

TABLE 1

Folic Acid Released after 2-hr Incubation at 37° C of a Mixture of Human Plasma and Takadiastase*

Plasma ml	Takadiastase mg	B_c released mμg
0.5	20	25
0.5	20 (boiled)	25
0.5 (boiled)	20	2
none	20	1.6
0.5	none	0

* Microbiological determination with *Str. faecalis*.

A careful study of the interaction between takadiastase and plasma, both in the native state and after boiling for 10 min, has led to opposite results (*4*). The plasma is deprived of B_c conjugate, but it contains a thermolabile enzyme, which releases folic acid from a conjugate. The role of takadiastase in this interaction is that of a substrate containing vitamin B_c conjugate in significant amounts. Folic acid is set free, see Table 1, after incu-